VACCINIA AND VARIOLA

A

STUDY OF THEIR LIFE HISTORY

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BY

JOHN B. BUIST, M.D., F.R.S.E.

FELLOW OF THE ROYAL COLLEGE OF PHYSICIANS, EDINBURGH;
PHYSICIAN TO THE WESTERN DISPENSARY; AND TEACHER OF VACCINATION FOR
THE LOCAL GOVERNMENT BOARD

Go to the quality of the active principle; abstract it from the material, and contemplate it by itself. Then determine the time; how long, at furthest, this thing, of this particular quality, can naturally subsist."—*Marc. Aurel.*, book ix. sect. 25.

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PREFACE.

THE causation and preventability of Infective Diseases have been the subject of much more careful and exact study since the discovery that a specific contagium of definite form is the cause of anthrax. The difficulty which is encountered in determining the cause of many infective diseases lies in the circumstance that no definite bacteric form peculiar to them can be isolated; and this is occasioned by the minute forms in which such contagia generally exist. Improved methods of histological investigation, by means of different staining materials and improved magnifying and illuminating apparatus, have now made it possible to define forms which were formerly beyond the range of human vision. The methods of pure cultivation of organisms, in liquid and solid media, employed respectively by Pasteur and Koch, have also greatly contributed to increase the knowledge of contagia. The method of pure cultivation of organisms in the living animal body, recommended by Koch as superior to all cultivating apparatus, must also be mentioned as showing the absolute necessity of experimental research in the investigation of infective diseases.

An attempt has been made in this work to determine the bacteric form in which the contagium of vaccinia and variola exists, in the materials which are capable of reproducing these diseases by inoculation; and, in aid of the inquiry, advantage has been taken of the most modern methods of bacteriological research. The bacteric forms assumed by cultivations of the materials employed have also been carefully studied and exactly delineated. Experimental vaccination appears to prove that the potency of vaccine materials varies in a remarkable degree, in proportion to the quantity and quality of their active principle. The probable origin of the virus, and the questions of immunity and attenuation, have also been the subject of investigation.

I take this opportunity of expressing my thanks to Dr George Buchanan and Dr Henry Stevens for much practical information regarding vaccine materials; and also to Professor Chiene, who has kindly granted me the free use of his Bacteriological Laboratory in the University of Edinburgh, during an extended period, for the purposes of this inquiry. The value of the observations is much increased by the fact that all the primary cultivations were made by his assistant, Mr A. W. Hare, who is a skilful and expert manipulator.

The illustrations of the work have been beautifully reproduced by Messrs Scott and Ferguson, from the original oil paintings by Mr C. K. Robertson, artist, and from the water-colours by Dr F. M. Caird and Mr W. Cathie.

Many able suggestions, and much assistance in the production of the work, have been received from my friends Dr Francis Troup and Mr C. K. Robertson.

My thanks are also due to Dr Birdwood, Mr Bott, Mr Clatworthy, and Dr Wood, for selecting the cases from which the cultivations and inoculations of small-pox were made; and also to Dr Cory, for assistance and information regarding animal vaccination.

I hope that the observations recorded may prove a useful guide to students, and to practitioners engaged in the practice of vaccination.

1 Clifton Terrace,
Edinburgh, 1*st September* 1887.

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LIST OF ILLUSTRATIONS.

ERRATA.

Pages 3, 28, *for* "innoculated" *read* "inoculated."
Page 8, *for* "innoculation" *read* "inoculation."
Page 29, line 7 from top, *for* "aseeptic" *read* "aseptic."
Page 43, 190, *for* "Blue" *read* "Bullæ."
Page 63, line 4, *for* "5 μ" *read* "·5 μ."
Page 63, line 3 from foot of page, *for* "·1 μ" *read* "1 μ."
Page 66, last line, *for* " m" *read* "from."
Page 72, *for* "of" *read* "or."
Page 82, side note, *for* "eruptions" *read* "eruption."
Page 86, *for* "II." *read* "2."
Page 94, Plate XVII., *for* "No secondary" *read* "Secondary."
Page 102, line 5 from foot of page, *for* "left" *read* "right."
Page 105, *for* "Plate II." *read* "Plate XVI."
Page 107, No. 51, *for* "No secondary" *read* "Secondary."
Page 127, *for* "·2 μ" *read* "2 μ."
Page 130, line 6, *for* "preparations" *read* "proportions."
Page 140, line 8, *for* "it" *read* "its."
Page 140, line 9, *for* "transferred" *read* "transformed."
Page 141, *for* "become" *read* "becomes."
Page 148, foot note 1, *for* "Gattingen" *read* "Gattungen."
Page 149, line 2 from foot of page, *for* "Swarm or "*read*" or Swarm-."
Page 174, line 7, *for* "inorganic" *read* "unorganised."
Page 223, *for* "Metabolism, theory" *read* "Metabolism theory."

LIFE HISTORY

OF

VACCINIA AND VARIOLA.

CHAPTER I.

THE VACCINE CONTAGIUM AND STANDARD VACCINE LYMPH.

Propagation of the vaccine contagium.

SINCE its discovery by Jenner, the propagation of the vaccine contagium, notwithstanding its mysterious nature, has been successfully accomplished in its purest form in the human body, by means of standard vaccine lymph. This mysterious virus is contained in the clear lymph, which is produced by typical Jennerian vesicles, and it is characterised by its peculiar power of reproducing the same disease, when transferred by inoculation from one person to another. When clear vaccine lymph is properly employed, the resulting vesicle is plump, round or oval, and pearl-coloured, with an elevated margin and a depression, or umbilication, in its centre; and it contains on the

Typical Jennerian vesicle.

eighth day, or even earlier, a material which is identical in appearance and potency with that by which it was originally produced.

On the eighth day, the vesicle becomes surrounded by a rose-coloured zone of inflammation or areola, which continues to increase for several days, and the clear contents of the vesicle are then found to be opalescent or opaque. The establishment of this areola is regarded as a proof of the specific effect of vaccination on the constitution. From the eighth to the tenth day, the opacity of the lymph continues to increase, after which coagulation of the contents of the vesicle takes place, with the gradual formation of a dry brown scab by the fourteenth day. This scab becomes harder, drier, and darker during the third week, when it falls off, leaving a depressed, circular or oval cicatrix, which is the natural termination of the process by which the majority of the human race are protected for a lifetime from small-pox. If such a termination to vaccination could be guaranteed with certainty, its beauty and simplicity could not be questioned.

Establishment of areola.

Termination.

Choice of material.

The material by which typical Jennerian vesicles are produced is clear vaccine lymph. It is, however, found in practice that, without various precautions, such uniform results are difficult to obtain, and great stress is accordingly laid by authorities on the proper selection of vaccine material. Thus, clear lymph is regarded by the authorities of the National Vaccine Establishment as a perfect material for vaccination, and they dis-

courage the use of opalescent lymph, which they regard as an imperfect vaccine material. The distinction between the two materials is at present arbitrary and empirical, which is a serious disadvantage in practice. The employment of perfect vaccine material, however, is found to be insufficient for the attainment of perfect vaccination in a great many cases, as various disturbing elements interfere with the result. Thus, the child to be vaccinated, though not positively unhealthy, may be flabby and generally below par, and such children are incapable of producing perfect material. In such cases the lymph is certain to be opalescent or opaque, while the vesicle is usually surrounded by an areola when the proper time comes for taking lymph. Vaccine lymph cannot be propagated successfully by means of unhealthy subjects. Choice of material.

The choice of the operation is also of great importance, and involves a knowledge of the relative potency of vaccine materials. The most perfect results are produced by the use of fresh lymph transferred directly from a typical vesicle on the arm of a healthy child to the arm of another. When innoculated with such material, four single scratches, one-third of an inch long, and three quarters of an inch distant from each other, will produce four good-sized vesicles, leaving cicatrices covering collectively at least half an inch square. If such lymph, however, be collected in a capillary tube, and used in the same manner half an hour afterwards, it will be found that only one, or perhaps two, of the Choice of operation. Fresh lymph.

Choice of operation.

insertions have been successful, and that the size of the vesicles is very much diminished. To obtain a result equal to the first, the lymph must be inserted into the arm by abrasion; at least forty light scratches being necessary. This method is imperative for the successful employment of lymph stored in any way whatever, because storage of lymph, even for the shortest period, exerts an injurious influence upon its activity, and a comparatively more severe operation is required. To prevent such failures, the directions issued by the Association for the Supply of Pure Vaccine Lymph should be strictly observed. "First wash the arm in clean water, and rub it briskly until dry with a clean towel. Blow the vaccine upon the arm, scratch through with a *new* needle or pin-point, taking care not to draw blood, and rub the vaccine well into the scratches with the eye of the needle, or the head of the pin-point." These directions are specially applicable to the use of stored calf lymph, dry or liquid, and stored human lymph, dry or liquid. When arm-to-arm lymph is employed as the vaccine material, total failure of a primary vaccination in good hands is exceedingly rare; but such vaccinators not unfrequently fail if they employ either stored calf or human lymph. When a primary vaccination fails, it should be a rule to repeat the operation by abrasion with arm-to-arm lymph only, because every vaccination, though apparently unsuccessful, exerts some constitutional influence, which can be only overcome by the employment of the most

Stored lymph, calf or human.

Failure of primary vaccination.

active material. When the primary vaccination has been only partially successful; *e.g.*, when one or two retarded vesicles, instead of four, are seen on inspection; Bryce's test should always be applied to ascertain whether the first vaccination is sufficiently protective. Even should no vesicles result, the protective effect of the primary vaccination is increased, as is shown by its subsequent progress. Bryce's test.

While the superiority of arm-to-arm lymph over any kind of stored lymph can be easily demonstrated, it is a much more difficult matter to determine whether dry lymph is superior or inferior to lymph stored in the liquid state. Dr Seaton, in discussing this point, states that he has found that the most skilful vaccinators prefer dried lymph, but that most vaccinators find it easiest to make lymph take which has been stored in capillary tubes. My own experience shows that dried lymph is undoubtedly superior as a vaccine material to fluid lymph stored in tubes. If changes take place in natural vaccine when stored in the liquid state, which do not occur when it is stored in a dry condition, the latter method of storage is evidently the superior of the two. This question is important as bearing upon the influence exerted by heat upon vaccine lymph. The method of storage is also important in relation to the problem whether the vaccine contagium can be grown artificially outside the animal body. If it can be proved, as I have no doubt it can, that standard Methods of storing lymph compared.

Constant character of material.

vaccine lymph contains a contagium vivum of constant character, it is evident that the preservation of this characteristic form, not only in the natural material, but also in artificial cultivations, is imperative. Sir James Paget remarks that "all pathological researches accumulate the evidences of the constant correspondence between the material in the blood on which each specific disease depends, and the morbid structure by which each is manifested. And so, if the vaccine virus were capable of any transformations besides those which mark its regular influence in each patient, such transformations, we may be sure, would be indicated by corresponding and evident changes in the vaccine vesicle. In other words, if the vaccine were changed into any other virus, there would be no vaccine vesicle."

Its active principle.

It must be confessed that nothing was known as to the composition of the material by which typical vaccine vesicles are produced, until the researches of Weigert, Klebs, Cohn, and Burdon Sanderson, rendered it probable, if not certain, that a "germ" was the active principle in vaccine lymph. A great impetus was given to this and other similar investigations by the previous researches of Pasteur on Fermentation, and Lister on Putrefaction of Wounds. Notwithstanding Cohn's exact research and beautiful delineation and description of the germ of variola and vaccinia, it is still an open question what vaccine lymph really is, and how it differs from opalescent or

opaque lymph. Considering the abundance of vaccine and variolous material, available both in the fresh and stored condition, this is a matter of surprise, and it can only be accounted for by the general satisfaction which exists with regard to the convenience and utility of Jenner's "matchless discovery." Such an inquiry involves the possession of special pathological training, and also extensive opportunities for both clinical and experimental research, and few practitioners have the requisite leisure, even with such opportunities and qualifications.

Its real nature doubtful.

My attention was first directed by difficulties encountered in procuring reliable vaccine material to the nature and cause of opacity in stored vaccine lymph. Is it a precipitate or a degeneration of the material? Is there a transformation of the active principle? Or is the opacity due to contamination from without? The answer to such questions is much more difficult than appears at first sight, involving as it does a review of all that is known with regard to smallpox and vaccination. The inquiry has thus extended itself far beyond the limits which were at first intended. In order to exclude the fallacies which might arise from accidental contamination of lymph, it became necessary to undertake a long series of experiments to determine, if possible, the nature and amount of germinal matter in commercial vaccine tubes. The explanation of the naked eye and histological appearances in clear and opaque vaccine lymph

Cause of opacity.

Extended inquiry necessary.

Cause of opacity.

was found, owing to staining difficulties, to be impossible without the employment of artificial cultivations. The results of microscopic examination of these were most difficult to explain, and the difficulty of explanation was increased by the failure to produce a local disease by means of experimental vaccination with cultivations. It was, therefore, found necessary to extend the inquiry to the contagion of smallpox, and to obtain a series of cultivations of that disease, and to compare these with vaccine cultivations. I have to acknowledge the kindness of Dr Birdwood, the Medical Superintendent, and Messrs Bott and Clatworthy, the Resident Physicians, in assisting me to obtain material for this purpose from cases in the Hospital Ships, Purfleet. The results of experimental innoculation with cultivations of variola have been most satisfactory, and have enabled me to draw conclusions as to the nature and cause of opacity in lymph.

Course of inquiry.

Its importance.

The determination of the essential nature of the vaccine contagium was found to be of the greatest importance the further the inquiry proceeded, and the possible relation of ordinary fermentation to vaccinia and variola has been considered as far as time and opportunity have allowed. The difficulty in arriving at the conclusions was much increased by the state of confusion at present existing with regard to the classification of Bacteria, and specially of the Sphæro-bacteria. I have adopted Cohn's classification as most elastic,

avoiding for the present newer classifications, which are only provisional.

Results.

The result of the inquiry has been an attempt to classify vaccine materials, natural and artificial, according to their composition and action, and to show the relation which the true vaccine contagium bears to artificial vaccine cultivations. The research has also appeared to me to throw some light upon the questions of immunity and attenuation of virus, and I have been able also to formulate some conclusions which, I believe, will be of service in practice.

CHAPTER II.

EXAMINATION OF EMPTY COMMERCIAL VACCINE TUBES.

Object of examination.

THE following experiments were undertaken with the view of ascertaining whether empty commercial vaccine tubes contain germinal matter which might give rise to opacity in lymph, and introduce a source of fallacy into the conclusions as to its cause.

Forty boxes of tubes were obtained for examination from Mr Somerville, Stockbridge, Edinburgh, and with these two series of experiments were made. The *first series* shows the action of sealed empty commercial vaccine tubes, sterilised externally, when introduced into beakers containing sterile nutrient fluid, and afterwards broken with aseptic precautions. The *second series* shows the behaviour of sterile and non-sterile nutrient fluid in commercial vaccine tubes and in sterilised vaccine tubes.

Two series of experiments.

FIRST SERIES OF EXPERIMENTS.[1]

First series.

Twenty beakers, plugged with cotton-wool, were

[1] *Cf.* Chiene, *Trans. Med. Chir. Soc, Edin.*, 1884; Tyndall, *Floating Matter of the Air.*

sterilised and charged, under spray, with 150 c.c. of sterile nutrient fluid (Darby's fluid meat in $\frac{1}{2}$ per cent. solution). First series of experiments.

Sixteen of the beakers so charged were, to ensure sterility, steamed for fifteen minutes on each of two consecutive days.

Four of the beakers, not so steamed, were kept under observation as a test of spray action in preserving sterility.

All of these beakers were then incubated at 35° C. for four hours on each of four successive days. A week afterwards the contents of all remained clear.

Empty commercial tubes having been sealed and sterilised externally by immersion in 1-20 solution of pure phenol, ten of these were then introduced (under spray, with hands washed in 1 per cent. solution of corrosive sublimate) into each beaker, and the beakers again incubated for four hours on each of four successive days. Seven beakers became cloudy.

The tubes in seven of the remaining beakers were then broken under spray with a sterilised glass rod. Those in the six others were left unbroken; and, as a control experiment, their plugs of cotton-wool were removed, and their contents stirred with a sterilised glass rod. Both of these latter sets of beakers were then incubated as before, with the following result:—

A.—Of beakers containing broken commercial tubes, Result.
Three remained clear,
Four became cloudy;

First series of experiments.

B.—Of beakers containing unbroken commercial tubes,

Five remained clear,
One became cloudy.

Thus, under the same conditions, four beakers out of seven containing broken commercial tubes became contaminated, while only one out of six beakers containing sealed unbroken empty commercial tubes did so. The first series of experiments was therefore strongly in favour of the view that *empty commercial vaccine tubes contain matter capable of germinating;* and we had next to determine the amount of such matter, and its probable influence on a nutrient fluid stored in them.

Result.

Second series.

Second Series of Experiments.

Exp. I.

Experiment I.

Here the aim was to ascertain the results when commercial and when sterilised vaccine tubes were similarly charged with sterile fluid under spray. The problems to be solved might be expressed shortly in an algebraic form :—

Problems.

Problem I.—Commercial vaccine tubes, (C.T.) + sterile fluid, (S.F.) + spray = ?

Problem II.—Sterilised vaccine tubes, (S.T.) + sterile fluid, (S.F.) + spray = ?

Under Problem I. ten (C.T. + S.F. + spray), preserved upright in a test-tube for three weeks, showed a floccu-

lent precipitate at the lower end of the column of fluid. Six weeks later, the precipitate was very distinct. Second series of experiments.

Under Problem II. ten (S.T. + S.F. + spray), preserved in a similar manner, showed no change after nine weeks.

The result of this experiment was, therefore, that commercial vaccine tubes, charged with sterile fluid under spray, showed precipitate; while sterile vaccine tubes similarly treated exhibited no change; or, in algebraic form— Exp. I. Results.

PROBLEM I.—Commercial vaccine tubes + sterile fluid + spray = precipitate.

PROBLEM II.—Sterile vaccine tubes + sterile fluid + spray = no change.

The sterility of the tube therefore makes some difference on the condition of the fluid stored in them.

Experiment II. Exp. II.

This experiment dealt with commercial vaccine tubes, charged with sterile fluid, but without spray.

PROBLEM.— Commercial vaccine tubes (C.T.) + sterile fluid (S.F.) − spray = ? Problem.

A hundred (C.T. + S.F. − spray), preserved upright in a test-tube for a week, showed a flocculent precipitate like an opaque ring supporting the column of fluid. This could be seen in all the tubes, and was very distinct when looked at by obliquely transmitted light; or

Second series of experiments.

by reflected light against a dark or shaded background. The tubes were taken from boxes 1 to 10.

Result.—Commercial vaccine tubes + sterile fluid − spray = precipitate.

Exp. III.

Experiment III.

Here we dealt with commercial vaccine tubes charged with doubtful sterile fluid, and without spray.

Problem.

PROBLEM.—Commercial vaccine tubes, (C.T.) + doubtful sterile fluid, (? S.F.) − spray = ?

A hundred commercial tubes (C.T., from boxes 11 to 20 + ? S.F. − spray), after three weeks, showed a precipitate like that in the preceding case. This experiment was conducted under conditions the same as those under which vaccine lymph is stored.

Result.

Result.—Commercial vaccine tubes + doubtful sterile fluid − spray = precipitate.

To prepare for the next experiments, twenty test-tubes (numbered 1 to 20) were charged with ten tubes each from the twenty boxes used for Experiments II. and III.; they were then plugged with cotton-wool and sterilised by heat.

Exp. IV.

Experiment IV.

Problem.

PROBLEM.—Sterilised vaccine tubes, (S.T.) + doubtful sterile fluid, (? S.F.) + spray = ?

The sixty sterilised tubes from test-tubes 1 to 6 (*i.e.*,

from boxes 1 to 6) were filled separately and laid on the table, while the test-tubes were being emptied. They were then sealed, the fluid being apparently sterile. The experiment took a considerable time. A fortnight afterwards, every one of these tubes showed a precipitate. Second series of experiments.

Result.—S.T. + ? S.F. + spray + time = precipitate. Exp. IV. Result.

Experiment V. Exp. V.

PROBLEM.—Sterilised vaccine tubes, (S.T.) + sterile fluid, (S.F.) + spray + speed = ? Problem.

The hundred sterilised tubes from boxes 7 to 20 (excepting boxes 8 and 12, *v.* Experiment VI.) were taken *en masse* from each of the test-tubes containing them; filled simultaneously by dipping their extremities in the fluid; and sealed without being laid on the table. The manipulation was much more rapid than in Experiment IV. Ten days afterwards, all these tubes remained clear.

Result.—S.T. + S.F. + spray + speed = no reaction. Result.

Test-tubes 8 and 12 were accidentally broken, and the twenty vaccine tubes they had contained were used for Experiment VI.

Experiment VI. Exp. VI.

PROBLEM.—Contaminated sterilised tubes, (C.S.T.) + non-sterile fluid, (N.S.F.) − spray = ? Problem.

Second series of experiments.

Twenty (C.S.T. + N.F. − spray) showed a very distinct precipitate eighteen days after having been filled. They were compared with the tubes used in Experiments II. and III. to determine whether there is any increase of the precipitate in proportion to the non-sterility of the fluid; and it was found that the precipitate was much more distinct when the fluid was non-sterile. The conditions of this experiment correspond exactly with those under which vaccine lymph is stored for use.

Exp. VI. Result.

Result.—C.S.T. + N.S.F. − spray = very distinct precipitate.

Exp. VII.

Experiment VII.

Problem.

PROBLEM. — Sterilised vaccine tubes, (S.T.) + sterilised fluid, (S.F.) − spray = ?

One hundred and ninety-two (S.T. + sterile hydrocele fluid − spray) showed, after eighteen days, a white precipitate at the lower end of the column of fluid. This experiment corresponds to the conditions under which vaccine lymph, supposing it or be a sterile fluid, is stored.

Result.

Result.—S.T. + S.F. − spray = white precipitate.

Microscopic examination of the precipitates in the foregoing experiments showed granules, angular bodies like crystals, and débris. No organisms of definite size and shape could be detected.

TABLE *of Second Series of Experiments.* Second series of experiments.

No. of Experiment.	Tubes.	Fluid.	With or without Spray.	Result.
I. *a.*	10 Commercial.	Sterile.	Spray.	Opacity.
I. *b.*	10 Sterile.	Sterile.	Spray.	Clear.
II.	100 Commercial.	Sterile.	No spray.	Opacity.
III.	100 Commercial.	(?) Sterile.	No spray.	Opacity.
IV.	60 Sterile.	(?) Sterile.	Spray + Time.	Opacity.
V.	100 Sterile.	Sterile.	Spray + Speed.	Clear.
VI.	20 Contaminated.	Non-sterile.	No spray.	Opacity.
VII.	192 Sterile.	Sterile.	No spray.	Opacity.

Summary.

Note.—Experiments III. and VI. correspond exactly to the conditions under which vaccine lymph is usually stored for use ; Experiments II. and VII. to the same, if vaccine lymph be supposed a sterile fluid.

Cultivations of precipitates.

Cultivations were made with the precipitates of two tubes from each experiment, but the tubes were not incubated, and no result of importance was obtained. There was an entire absence of reaction, except in one instance, where minute white "cocoons"[1] were obtained; this result may have been due to contamination.

GENERAL CONCLUSIONS.

General conclusions.

It appears to be certain that commercial vaccine tubes contain germinal matter, but that such material exists in very small amount in tubes which (as in Somerville's boxes) have been kept carefully protected from dust after being drawn. The experiments detailed point, however, to the expediency of sterilising

[1] See p. 25.

General conclusions.

the tubes before using them: sterility of the tubes, sterility of the fluid, and the use of the spray are all necessary to prevent opacity from taking place in the fluid.

Sterilisation of tubes.

The sterilisation of the tubes is best done by immersing them in alcohol or ether, and then superheating them in test-tubes, plugged with cotton-wool, in a hot-air chamber. An ordinary oven answers this latter purpose very well. Carbolic acid solution should not be used, as microscopic crystals, which would afterwards exert a paralysing or germicidal influence on the vaccine lymph, may be left in the interior after drying. Dougall showed, indeed, that this paralysing effect of carbolic acid was only temporary, as the vaccine lymph slowly regains its power when the acid evaporates; but such evaporation cannot take place in sealed tubes.

Opacity.

I have found that opacity appears sooner in lymph stored in commercial tubes than in that stored in sterilised tubes, and that it is greater in amount: clear vaccine lymph, if stored in sterilised tubes, shows only slight opalescence, and that after some time. The fact, however, that in the first series of experiments so large a proportion of the beakers containing broken tubes showed no change in the fluid, led to the conclusion that the amount of germinal matter in commercial tubes was very small. Further, in the second series, the amount of opacity in the fluid was very much less than that observed in vaccine lymph;

sterile tubes, as well as commercial ones, when charged without spray showed opacity; and the use of non-sterile fluid without spray very much increased such opacity. Accordingly, these observations incline me to agree with the opinion of Mr Farn, of the National Vaccine Establishment, "that opacity of lymph is due in a very small degree, if at all, to impurities in the tubes." General conclusions.

CHAPTER III.

EXAMINATION OF VACCINE CULTIVATIONS.

SECTION I.

Culture Methods and Media.

Culture methods.

A preliminary examination of clear and opaque vaccine lymph led to no definite conclusions either as to the presence of organisms or as to the best methods of staining them; but further observation has shown that special care has to be employed in staining lymph. No such difficulty is to be encountered in the case of cultivations, as the organisms are readily stained by aniline dyes.

As several organisms of different characters had been already cultivated by different observers, it was decided to attempt to vaccinate aseptically. The skin of the vaccinifer was first purified by washing the surface of the vesicle and the surrounding skin with 1-20 pure phenol and ether, and the vesicle was then opened with a lancet sterilised by heat. The arm of the infant to be vaccinated was similarly treated with ether and phenol, and the operation completed under

spray. The result of these precautions was a dismal failure, as nearly every one of the insertions was unsuccessful, and the few successful vesicles were much retarded in their progress on the eighth day. It was thus found that it was best to select typical cases for the purpose of cultivation, choosing healthy subjects bearing plump well-formed vesicles without areola. Culture methods.

Culture Media.

Four different culture media were at first tried:— Culture media.

1. Sterilised boiled potato;
2. Sterilised nutrient gelatine (Koch's);
3. Sterilised agar agar gelatine;
4. Sterilised hydrocele fluid.

Cultivations in hydrocele fluid and on boiled potato were not continued; the growth on potato was found to be hard and woody, and the liquid medium was abandoned because of the known difficulty of obtaining pure cultivations in the event of there being a mixture of organisms such as was anticipated.[1]

The naked-eye appearances of cultivations in agar agar were found to be indistinct and unreliable, the form

[1] Quist's culture fluid, however, appears to be perfectly suitable. It is composed of blood-serum of the ox, glycerine, and distilled water, —of each 100 parts,—with one part of carbonate of potash (*Berlin Klin. Wochensch.*, No. 52, 1883). Müller's method of storing and multiplying lymph, described in Ziemssen's *Cyclopædia*, may also be mentioned here. He finds that one part of clear vaccine lymph, mixed with two parts of glycerine and two of distilled water, is an effective material for vaccination. Is this not a pure cultivation in miniature?

Culture media.

and colour of the growth being especially indefinite, while those in Koch's nutrient gelatine were ultimately found to be perfectly definite and satisfactory. Pure cultivations to any extent could, in fact, be easily obtained by Koch's method. Such cultivations should not be incubated, as the gelatine liquefies.

SECTION II.

Macroscopical Appearances of Vaccine Cultivations.

Vaccine cultivations.

Source No. I.

No. in Register—447. Result—Four vesicles without areola. Material—Clear lymph, accepted by the National Vaccine Establishment. Date of Cultivation—May 18, 1885.

The vesicles were opened under carbolic spray, with a lancet sterilised by heat; and test-tubes Nos. 1, 2, 5, 7, and 8[1] were inoculated by Mr Hare with a sterilised platinum öze. Cultivations in sterilised hydrocele fluid and boiled potato were made at the same time as those in agar agar and Koch's nutrient gelatine.

Primary Cultivations in Agar Agar.

Primary.

In Nos. 1, 2, and 5 the growth was first observed after one day's incubation, the fourth from inoculation, when there was a triangular white film in the track of

[1] These numbers refer to the table of cultivations, p. 23.

TABLE *showing Macroscopical Appearances of Vaccine Cultivations.*

Table of vaccine cultivations.

No.	Name of Tube.	4th Day.	8th Day.	Result.
1	I.A, agar.	White.	Faint yellow.	Dull yellow.
2	I.B, ,,	,,	,,	,,
3	II.A, ,,	,,	Yellowish-green.	Bright yellow.
4	II.B, ,,	,,	,,	,,
5	I. ,,	No report.	No report.	Dull orange.
6	II. ,,	,,	,,	White and yellow.
7	I. jelly.	,,	Or., white, yel.	Orange.
8	I.B, ,,	,,	,,	,,
9	II.A, ,,	,,	White and yel.	Yellow.
10	II.B, ,,	,,	,,	,,
11	II. ,,	,,	No report.	,,
12	I.A′, agar.	Secondary.	,,	Orange.
13	II.B′, ,,	,,	,,	Yellow and white.
14	I.B′, jelly.	,,	,,	Bright orange.
15	II.A′, ,,	,,	,,	Yellow and white.
16	I. jelly, yellow.	,,	Orange.	Orange.
17	I. jelly, white.	,,	,,	,,
18	III.B, agar.	White.	Dull orange.	Dull orange.
19	III.A, ,,	,,	White.	White.
20	III.A, jelly.	No reaction.	No reaction.	No reaction.
21	III.B, ,,	,,	,,	,,
22	IV.A, agar.	From opa	que lymph.	,,
23	IV.B, ,,	,,	,,	,,
24	IV.A, jelly.	,,	,,	,,
25	IV.B, ,,	,,	,,	,,
26	V.A, agar.	White.	White.	White.
27	V.B, ,,	,,	,,	,,
28	V.A, jelly.	No report.	No report.	White and yellow.
29	V.B, ,,	,,	,,	,,
30	VI.A, agar.	Greyish-blue.	Greyish-white.	Thick wh. growth.
31	VI.B, ,,	,,	,,	,,
32	VI.A, jelly.	White.	White.	Jelly liquefied.
33	VI.B, ,,	,,	,,	Yellow.
34	VII.A, jelly.	,,	,,	White.
35	VII.B, agar	White.	Dull orange.	Dull or. & brown.
36	VIII.A, agar.	Wh. & dull or.	White.	Strong wh. growth.
37	VIII.B, agar.	,,	Dull orange.	Dull orange.
38	IX.A, jelly.	White.	White and yel.	White and yellow.
39	IX.B, agar.	,,	White.	Yellow.
40	X.A, agar.	,,	,,	White and yellow.
41	X.B, ,,	,,	White & dull or.	,,
42	XI. agar.	,,	White.	Wh., yel., & brown.

Vaccine cultivations.

the wire. The film was diffuse, and numerous minute masses were scattered through it.

On the *eighth day*, No. 1 showed, on the surface, an opaque growth, partly bluish-grey and partly faint yellow. Below the surface, the growth was greyish-white, extremely dense and diffuse, and the minute scattered masses could not be distinguished. Deeper in the agar, the dense growth occupied the centre, and was surrounded by the minute white masses. At the end of *seven weeks*, there was a yellow growth on the

Primary.

surface, surrounded by a bluish-grey film; and, at the end of *three months*, the growth was still yellow.

In No. 2, on the *eighth day*, there was a bluish-grey film on the surface, with a yellow growth in the centre. The diffuse growth was not nearly so thick as in No. 1, and the small grey or white masses could be easily distinguished deep in the agar. At the end of *seven weeks*, there was a yellow and dull orange or brown growth on the surface. At the end of *three months*, there was a thick white disc intermingled on the surface with dull orange.

I have no notes of No. 5 in the early stages, but it ultimately showed a grey film with dull orange colour on the surface.

Primary Cultivations in Koch's Nutrient Gelatine.

Primary.

No. 7, on the *eighth day*, showed a circumscribed orange growth on the surface. Below, were separate

white and yellow oval masses, sharply defined, and of various sizes; and there was no diffuse film between the masses. I have named these oval growths "cocoons." Vaccine cultivations.

No. 8, on the *eighth day*, exhibited an orange growth on the surface; below, there were distinctly separated white and yellow cocoons.

Seven weeks afterwards, both Nos. 7 and 8 showed a large orange growth on the surface. *Three months* afterwards, No. 8 had become contaminated by mould.

Secondary Cultivations.

From No. 1, of a dull yellow or orange colour, test-tube No. 12, containing agar agar, was inoculated. *Three weeks* afterwards, there was a dull orange growth on the surface. After *seven weeks*, there was, on the surface, a bluish-white film with a pale orange patch in its centre. Beneath the surface, there was a whitish cloud with minute orange cocoons in it. Secondary.

From No. 8, an orange growth in jelly, test-tube No. 14 was inoculated. *Three weeks* afterwards the growth was bright orange. It was circumscribed, sharply defined, and from one-eighth to one-sixth of an inch thick.

Test-tube No. 8 was broken at the bottom, and an attempt was made to obtain pure cultivations of the white and yellow cocoons which were embedded deeply in the gelatine. No. 16 was inoculated from one of the yellow cocoons, and No. 17 from one of the white

Vaccine cultivations.

cocoons. In both, *eleven days* afterwards, an orange growth appeared on the surface.

Secondary.

Further pure cultivations of the orange growths showed a diminution in the brightness of the colour; in the agar agar cultivations the colours were dull, and difficult exactly to define.

Source No. II.

No. in Register—446. Result—Four vesicles without areola. Material—Clear lymph, accepted by the National Vaccine Establishment. Date of Cultivation—May 18, 1885.

The same precautions were observed in making the cultivations as were used in those from Source No. I.

PRIMARY CULTIVATIONS IN AGAR AGAR.

Primary.

On the *fourth day*, No. 3, after incubation at 35° C. for four hours, showed about twenty small rounded white growths on the surface and in the track of the wire. These were commencing to show an uncertain tinge of colour; and there was no diffuse opacity between the masses.

No. 4 showed two similar white growths.

On the *eighth day*, No. 3 showed, on the surface, two pale yellowish-green masses; for half an inch below the surface a thin semi-transparent white film was growing, containing very minute masses scattered through it.

One edge of this film was occupied by masses of much larger size, those nearer the surface being pale greenish-yellow, and the others white; these were about twenty in number, and not connected by any diffuse film. After *seven weeks* there was, on the surface of the agar, a bluish-grey film with a yellow growth in the centre. Beneath the surface some of the cocoons had become dull orange; the others were yellow. *Three months* afterwards there was, on the surface, a strong yellow growth. Vaccine cultivations. Primary.

No. 4, on the *eighth day*, showed two masses, the surface growth being yellowish-green, and the deeper one greyish-white. After *seven weeks* there was on the surface a thick yellow growth, which had spread strongly after *three months*.

Primary Cultivations in Koch's Nutrient Gelatine.

Nos. 9 and 10, on the *eighth day*, showed on the surface a yellow growth, beautifully circumscribed; below the surface the growth was white. Primary.

After *seven weeks*, No. 9 showed a large yellow growth, with numerous surface depressions, probably due to evaporation. The growth projected downwards into the substance of the jelly like a mould, and its colour was paler at the circumference than in the centre. Below the surface, there were two large yellow, coral-like growths, connected by a lace-like

Vaccine cultivations.

tracery. These consisted of aggregated cocoons. After *three months*, evaporation of the gelatine had taken place, and the growth adhered partly to the sides of the test-tube.

Primary.

Nos. 10 and 11, after *seven weeks*, showed a yellowish growth on the surface, and a yellow cocoon below.

Secondary Cultivations.

Secondary.

From No. 4, a pale greenish-yellow, and white growth, No. 13 was innoculated. *Three weeks* afterwards, there were pale yellow and white cocoons, and after *seven weeks*, the growth was yellow, both on and below the surface.

From No. 9, a yellow and white growth, No. 15 was inoculated. *Three weeks* afterwards, there was on the surface a bright yellow growth, apparently made up of flattened cocoons, and with white cocoons below. After *seven weeks*, the colour on the surface was still yellow, with small white cocoons below.

Further pure cultivations were easily made from yellow cultivations, the only change being an increasing dulness of the colour in proportion to the age of the cultivations.

On potato and in hydrocele fluid.

The cultivations from Sources Nos. I. and II., made on boiled potato, showed a white, yellow, and orange growth, dry and hard in character. They were rejected as being unsuitable. The cultivations from the same sources in hydrocele fluid exhibited a white

flocculent precipitate, which was also rejected as being indefinite. Vaccine cultivations.

Source No. III.

No. in Register—477. Result—Four vesicles without areola. Material — Clear lymph. Date of Cultivation—June 18, 1885.

Minute asceptic precautions were employed in making the cultivations.

Primary Cultivations in Agar Agar (Nos. 18 and 19).

After *three days'* incubation, at 35° C. for four hours each day, No. 19 showed a faint white cloudy growth. On the *eighth day*, there was one white cocoon in the cloud. At the end of *a month*, the growth was cloudy and white, and after *three months* it showed no change. Primary.

No. 18 showed, on the *fourth day*, a luxuriant white growth in the track of the wire, there being a diffuse cloud in the centre, surrounded by numerous white nodules. On the *eighth day*, there was on the surface a greyish-blue growth; below, the growth was white, mingled with a dull orange or brown colour. At the end of *a month*, the growth was dull orange on the surface, and had dull-brown cocoons below. After *three months* there was no further change.

Cultivations in Koch's gelatine were unsuccessful.

Vaccine cultivations.

Source No IV.

No. in Register—480. Result—Four vesicles surrounded by areola. Material—Opaque lymph. Date of Cultivation—June 18, 1885.

Unsuccessful.

Cultivations in Agar Agar and Koch's gelatine were unsuccessful. There was no contamination of the media.

Source No. V.

No. in Register—478. Result—Four vesicles without areola. Material—Clear lymph. Date of Cultivation—June 18, 1885.

Primary Cultivations in Agar Agar.

Primary.

No. 26, after *three days'* incubation, showed in the track of the wire a luxuriant white growth, surrounded by minute white nodules, and having greyish-blue dots on its surface. After *eight days*, the white colour on the surface changed to grey; *a month* afterwards, there was a white growth with minute cocoons, and after *three months* there was no further change.

Primary Cultivations in Koch's Nutrient Gelatine.

No. 28, after *a month*, showed a white growth on the surface, with separate white and yellow cocoons in the substance of the jelly. No. 29 also showed a white

growth on the surface; it had beautifully circumscribed white and yellow oval cocoons below. Vaccine cultivations.

Three months after, the growths were larger, but the colours were unchanged.

Source No. VI.

No. in Register—475. Result—Four broken vesicles with areola. Material—Clear lymph, (?). Date of cultivation—June 18, 1885.

Primary Cultivations in Agar Agar (Nos. 30 and 31).

No. 30, after *three days'* incubation for four hours each day, showed the surface thickly covered by a greyish-blue film. Cocoons were seen below, but the general appearance of the growth was obscured by cloudiness. On the *eighth day* the surface-growth was a thick greyish-white, with cloudy growth below. *A month afterwards*, the white growth on the surface appeared to be contaminated. *Three months* afterwards, there was on the surface a thick white disc. Primary.

No. 31, after *a month's* growth, showed a thick white disc on the surface, which became brown after three months.

Primary Cultivations in Koch's Nutrient Gelatine.

No. 32 showed white cocoons on the *fourth day*

Vaccine cultivations.

both on the surface and below. On the *eighth day*, there was a well-marked white growth, both on the surface and in the track of the wire; below the surface, there were white cocoons about the size of a mustard seed. After *three months*, liquefaction of the jelly had taken place. The precipitate was white.

Primary.

No. 33 showed a white growth on the *fourth* and the *eighth* day, which had become yellow after *three months'* growth.

Source No. VII.

No. in Register—479. Result—Four vesicles with areola. Material—Opalescent lymph. Date of Cultivation—June 22, 1885.

Primary Cultivation in Koch's Nutrient Gelatine.

Primary.

No. 34 showed commencing white cocoons on the *fourth day*, which had increased in size on the *eighth day*. *Four weeks* afterwards, the growth was still white, and at the end of *three months* the jelly had liquefied.

Primary Cultivation in Agar Agar.

Primary.

No. 35 showed a white growth on the *fourth day*, which had changed to dull orange or brown on the *eighth day*. After a *month*, the colour of the growth

was dull orange, and after *three months* there was a brown growth on the surface. Vaccine cultivations.

Source No. VIII.

No. in Register—483. Result—Four vesicles without areola. Material—Clear lymph. Date of Cultivation—June 22, 1885.

PRIMARY CULTIVATIONS IN AGAR AGAR.

Nos. 36 and 37 showed, on the *fourth day*, a dull orange or brown growth on the surface, with a white growth below. Primary.

On the *eighth day*, No. 36 was white and slightly brown on the surface. After a *month's* growth, the brown colour had disappeared and the colour on the surface was white. There was no change after *three months*.

On the *eighth day*, No. 37 showed a dull orange growth on the surface, but the tube became contaminated by mould after a *month's* growth.

Source No. IX.

No. in Register—481. Result—Four vesicles without areola. Material—Clear lymph. Date of Cultivation—June 22, 1885.

PRIMARY CULTIVATION IN KOCH'S NUTRIENT GELATINE.

No. 38 showed a faint trace of white growth on

Vaccine cultivations. the *fourth day*, which was still white on the *eighth day*.

Source IX. After a *month's* growth, a tinge of yellow appeared; and it was distinctly white and yellow, both on the surface and in separate cocoons below it, after *three months*.

Primary Cultivation in Agar Agar.

Primary. No. 39 showed a white growth on the surface and below it, on the *fourth day*, which was still white on the *eighth day*.

A *month* afterwards the white growth had become yellow; and after *three months* there was a strong yellow growth.

Source No. X.

No. in Register—485. Result—Four vesicles surrounded by areola. Material—Opalescent lymph. Date of Cultivation—June 22, 1885.

Primary Cultivations in Agar Agar.

Primary. Nos. 40 and 41 showed, on the *fourth day*, a luxuriant white growth both on the surface and below it. On the *eighth day*, No. 40 showed a white growth; but in No. 41 there was a white growth with a tinge of colour. A *month* after, No. 41 showed a white growth changing

to yellow, while No. 40 was still white. After *three months'* growth, both Nos. 40 and 41 showed a white and yellow growth. Vaccine cultivations.

Source No. XI.

No. in Register—487. Result of Vaccination—Four vesicles without areola. Material—Clear lymph. Date of Cultivation—June 22, 1885.

CULTIVATION IN AGAR AGAR.

No. 42 showed a white growth on the *fourth day*, which had increased on the *eighth day*. After a *month*, the growth was still white; and after *three months*, it was white, yellow, and brown on the surface. Primary.

SECTION III.

ANALYSIS OF TABLE OF VACCINE CULTIVATIONS.

An examination of the table shows that the colour of the growth on the *fourth day* was white, with scarcely any exception. The exceptions (Nos. 30, 31, 36, and 37) were seen in primary cultivations in agar agar, which is not a good medium for displaying delicate variations of colour; and I experienced some difficulty in describing the exact shade. But even in this unfavourable medium the colour was white on the *fourth* Analysis of table of vaccine cultivations. Fourth day.

Analysis of table of vaccine cultivations.

day. The appearance of primary cultivations in Koch's nutrient jelly was invariably white on the *fourth day*.

Eighth day.

On the *eighth day*, half of the primary cultivations in agar agar were still white; while the other half had become yellow or yellowish-green, and dull orange or brown. Some of the primary cultivations in Koch's jelly were still white; others were white and yellow; while, in one instance, white, yellow, and orange colours appeared. *Subsequently*, primary cultivations in agar agar showed either a distinct white or a distinct yellow growth; and a few showed a white and yellow, or a white and dull orange or brown colour.

Subsequently.

Primary colours. White. Yellow. Orange. Brown.

In Koch's jelly, the colours were chiefly white and yellow, and only one or two showed a distinct pure orange colour. Three of the primary cultivations in agar agar (Nos. 35, 37, and 42) showed a brown colour. This brown colour appears to correspond to the orange colour in Koch's jelly. The orange and brown colours invariably occurred on the surface of the growth exposed to the air, while the white and yellow colours appeared also in the masses growing beneath the surface. Certain of the tubes containing primary cultivations were opened for the purpose of making microscopic preparations, and from these secondary cultivations were made at the time. No. 12, from No. 1, a yellow growth in agar, produced a dull orange cultivation. No. 13, from No. 4, a yellowish-green growth in agar, produced a white and yellow cultivation. No. 14, from No. 8, a white, yellow, and orange growth

Pure cultivations.

Colours.

in Koch's jelly, produced a bright orange cultivation only. No. 15, from No. 9, a white and yellow growth in Koch's jelly, showed no change. No. 16, from No. 7, a yellow cocoon in Koch's jelly, produced a bright orange cultivation. The original cultivation, No. 7, showed an orange growth on the surface, and separate white and yellow cocoons below it. No. 17, from No. 7, a white cocoon below the surface in the same test-tube, also produced an orange cultivation. Contamination with the orange growth on the surface was avoided by chipping a piece out of the bottom of the test-tube and withdrawing the culture materials by different tracks through the aperture.

Analysis of table of vaccine cultivations.

Pure cultivations.

Colours.

Change of colour during growth was thus observed to take place both in primary and secondary cultivations, and the question arose whether this change was due to growth of a mixture of organisms, or whether it occurred during the natural process of growth of a single organism. Further pure cultivations, made by Mr Hare, showed that the white, yellow, and orange growths can easily be cultivated separately. But even in these change occurs; a tinge of colour appears in the white, while the yellow and orange pure cultivations lose their brightness, and become duller in hue. As it is maintained by some bacteriologists that the colour of a micro-organism is distinctive of species, we must conclude provisionally from naked-eye examination that the cultivations detailed show the mode of growth of three separate germs. To these must be added a fourth,

Change of colour during growth.

Provisional conclusion.

Analysis of table of vaccine cultivations.

viz., the brown growth described in cultivations in agar. None of the organisms produces liquefaction of Koch's gelatine.

The primary cultivations were made by Mr Hare, with aseptic precautions, from clear vaccine lymph, and contamination was prevented. That is to say, the growths were produced by the organisms pre-existing in the lymph, just as an ordinary vaccination produces a vaccine vesicle. The lymph was taken from eleven different sources and an analysis from this point of view is very instructive.

Colours from different sources.

Source No. I. = Nos. 1, 2, 5, 7, 8, 12, 14, 16, 17.

Here the most definite colour after the *eighth day* was orange in Koch's jelly, and dull orange or brown in agar.

Source No. II. = Nos. 3, 4, 6, 9, 10, 11, 13, 15.

Here the prevailing colour was yellow, but three tubes showed white and yellow.

Source No. III. = Nos. 18, 19, 20, 21.

We got here a white and dull orange growth in agar agar. No growth appeared in Koch's gelatine.

Source No. IV. = Nos. 22, 23, 24, 25.

Gave no reaction. The lymph was opaque, and taken from a vesicle surrounded by areola.

Source No. V. = Nos. 26, 27, 28, 29.

Analysis of table of vaccine cultivations.

The cultivations from this source were white. Yellow cocoons appeared in Koch's jelly.

Source No. VI. = Nos. 30, 31, 32, 33.

Here white was the prevailing growth, but one white growth became yellow.

Source No. VII. = Nos. 34, 35.

Here the colours in agar agar were white and brown or dull orange.

Colours from different sources.

Source No. VIII. = Nos. 36, 37.

Here the colours in agar agar were again white and dull orange or brown.

Source No. IX. = Nos. 38, 39.

White and yellow colours in agar agar and Koch's gelatine were produced.

Source No. X. = Nos. 40, 41.

White and yellow colours in agar agar were again produced.

Analysis of table of vaccine cultivations.

Source No. XI. = No. 42.

Sources of colours.

White, yellow, and brown colours were produced in agar agar. Here the original colour was white, which continued *three weeks* without change. *Three months* afterwards the three typical colours in agar agar were present.

Result.

The result shows that white and yellow growths are common to both Koch's jelly and agar agar, the bright orange growth is only found in Koch's nutrient material, and the corresponding colour of the growth in agar is brown or dull orange.

CHAPTER IV.

EXAMINATION OF VARIOLOUS CULTIVATIONS.

SECTION I.

Source I.

Variolous cultivations.

CASE A.—J. M., female; 22 years. Early pustular stage. Admitted to Hospital Ships, September 12, 1885. No visible marks of vaccination. Date of cultivation—September 14, 1885.

CULTIVATIONS IN KOCH'S NUTRIENT GELATINE.

Showed no reaction.

CULTIVATION IN AGAR AGAR.

Primary.

Nos. 5 and 6 showed no reaction. Nos. 7 and 8 showed a trace of white growth after *two months*.

CULTIVATIONS IN SOLID SERUM.

Nos. 9 and 10 showed a white growth. The results from this case were meagre and unsatisfactory.

Variolous cultivations.

TABLE *showing the Macroscopical Appearances of Cultivations of Variolous Lymph in Solid Media.*

Source.	No.	Name of Tube.	8th day.	13th day.	Result.
Early pustular or pustular.	1	Aa, jelly.	No reaction.	No reaction.	No reaction.
	2	Ab, ,,	,,	,,	,,
	3	Ac, ,,	,,	,,	,,
	4	Ad, ,,	,,	,,	,,
	5	Ae, agar.	,,	,,	,,
	6	Af, ,,	,,	,,	,,
	7	Ag, ,,	,,	,,	White.
	8	Ah, ,,	,,	,,	,,
	9	Ai, serum	White riband.	White.	,,
	10	Aj, ,,	No reaction.	Trace of white.	,,
Blood, papulo-vesicular.	11	Ba, jelly.	White cocoon.	White cocoon.	Pure white.
	12	Bb, ,,	,,	,,	,,
	13	Bc, ,,	No reaction.	No reaction.	No reaction.
	14	Bd, Agar.	White cloud.	White cloud.	White cloud.
	15	Be, ,,	,,	,,	,,
	16	Bf, ,,	No reaction.	No reaction.	No reaction.
	17	Bg, serum	,,	,,	,,
	18	Bh, ,,	,,	,,	,,
Papulo-vesicular. Vesicular.	19	Ca, agar.	White zone.	White zone.	White zone.
	20	Cb, ,,	White cloud.	White cloud.	White cloud.
	21	Cc, ,,	White zone.	White zone.	White zone.
	22	Cd, serum	White cloud.	White cloud.	White cloud.
	23	Ce, ,,	,,	,,	,,
	24	Da, jelly.	White cocoons.	White.	White.
	25	Db, ,,	Liquefaction, and white Precipitate.		
	26	De, ,,	No reaction.	No reaction.	No reaction.
	27	Dd, agar.	White.	White.	Faint white.
	28	De, ,,	No reaction.	,,	White.
	29	Df, ,,	,,	No reaction.	No reaction.
	30	Dg, serum	,,	,,	,,
	31	Dh, ,,	White riband.	White.	White.

Cultivations of Variolous Lymph—continued.

Source.	No.	Name of Tube.	8th Day.	13th Day.	Result.
Vesicular.	32	Ea, jelly.	No reaction.	No reaction.	No reaction.
	33	Eb, ,,	White cocoon.	White cocoon.	White.
	34	Ec, ,,	,,	,,	Liquefying.
	35	Ed, ,,	,,	White, liquefying	with white p.p.
	36	Ee, ,,	,,	White.	White.
	37	Ef, ,,	Cocoons and	liquefying globe,	with white p.p.
	38	Eg, ,,	,,	,,	,,
	39	Eh, ,,	White cocoon.	Larger white.	Pure white.
	40	Ei, ,,	No reaction.	Small cocoon.	White.
	41	Ej, ,,	White cocoon.	Globe of liquefac.,	with orange p.p.
	42	Ek, ,,	,,	White.	Faint white.
	43	El, ,,	Cocoons.	Globe of liquefac.,	with yel. p.p.
	44	Em, agar.	White cloud.	White cloud.	White cloud.
	45	En, ,,	White zone.	Thick white zone.	White.
	46	Eo, ,,	Liquefying.	Liquefying.	Liquefying.
	47	Ep, ,,	White cloud.	White.	White.
	48	Eq, ,,	,,	,,	,,
	49	Er, ,,	Liquefying?	Liquefying?	Solid white.
	50	Es, ,,	White cloud.	White cloud.	Wh. with or.
	51	Et, ,,	No reaction.	No reaction.	No reaction.
	52	Eu, serum	White.	White.	White riband.
	53	Ev, ,,	No reaction.	No reaction.	No reaction.
Papulo-vesicular.	54	Fa, jelly.	Faint white.	White.	Faint white.
	55	Fb, ,,	Wh. cocoons.	,,	,,
	56	Fc, ,,	,,	,,	Wh. cocoons.
	57	Fd, ,,	No reaction.	No reaction.	No reaction.
	58	Fe, ,,	White.	White.	Liquefac. and white p.p.
	59	Ff, ,,	,,	,,	Faint growth.
	60	Fg, agar.	,,	Thick white.	White cloud.
	61	Fh, ,,	,,	,,	,,
	62	Fi, ,,	,,	,,	,,
	63	Fj, ,,	No reaction.	No reaction.	No reaction.
	64	Fk, ,,	,,	,,	,,
	65	Fl, serum.	White riband.	White riband.	White riband.
	66	Fm, ,,	,,	,,	,,
Blue.	67	Ga, jelly.	Funnel-shaped	liquefaction, with	white p.p.
	68	Gb, ,,	,,	,,	,,
	69	Gc, ,,	,,	,,	,,
	70	Gd, ,,	,,	,,	,,
	71	Ge, agar.	Or. or ochre.	Cocoons solid.	Ochre cocoons.
	72	Gf, ,,	,,	,,	,,
	73	Gg, ,,	,,	,,	,,
	74	Gh, ,,	White cloud.	White cloud.	White cloud.
	75	Gi, ,,	White and or.	White and ochre.	Wh. and or.

and *fourteenth days*, which was unchanged two months afterwards. Variolous cultivations.

No. 16 showed no reaction.

Cultivations in Solid Serum.

Nos. 17 and 18 showed no reaction.

Source III.

Case C.—A. L., female; 16 years. Pustular stage. Admitted to Hospital Ships, September 10, 1885. Vaccinated, only one mark visible. Date of cultivation —September 14, 1885.

Primary Cultivations in Agar Agar.

Nos. 19, 20, and 21 showed, on the *eighth* and *fourteenth* days, an opaque white zone on the surface of the gelatine. Primary.

The cultivations showed no change *two months* afterwards.

Cultivations in Solid Serum.

Nos. 22 and 23 showed large white cloudy growths on the *eighth* and *fourteenth* days, which were unchanged after *two months*.

Source IV.

Case D.—A. W., male; aged 5 years. Papulo-

Variolous cultivations. Source IV.

vesicular stage. Not vaccinated, but vaccinated on admission. Date of cultivation—September 14, 1885.

Primary Cultivations in Koch's Nutrient Gelatine.

Primary.

No. 24 showed small white cocoons on the *eighth* and *fourteenth days*, without liquefaction of the jelly. *Two months* afterwards the growth was unchanged.

No. 25 showed, on the *eighth day*, several cocoons, and an oval liquefied globe about the size of a pea, in the substance of the jelly, with a white precipitate at the bottom. On the *fourteenth day* the liquefaction had increased, and *two months* afterwards the jelly was liquefied, and the white growth had sunk to the bottom of the test-tube.

No. 26 showed no reaction.

Primary Cultivations in Agar Agar.

Primary.

No. 27 showed a small white nodule on the *eighth day*, which was small and indefinite after *two months'* growth.

No. 28 showed no reaction on the *eighth day*, but on the *fourteenth day* there was a cloudy white growth on the surface of the gelatine, which still existed, with a trace of growth below in the track of the wire, *two months* afterwards.

No. 29 showed no reaction.

Cultivations in Solid Serum.

Variolous cultivations.

No. 30 showed no reaction, but No. 31 showed, on the *eighth day*, a long white riband-shaped growth, which was still white *two months* afterwards.

Source V.

Case E.—T. A., male; 27 years. Pustular stage. Admitted to Hospital Ships, September 9, 1885. Vaccinated. Date of cultivation—September 14, 1885.

Primary Cultivations in Koch's Nutrient Gelatine.

No. 32 showed no reaction.

Nos. 33 and 34 showed a small white cocoon growth on the *eighth* and *fourteenth days*. In No. 34 the gelatine was liquefying after *two months'* growth. Primary.

No. 35 showed a white oval or cocoon growth on the *eighth day*, which was much larger on the *fourteenth day*. *Two months* afterwards there was a liquefying globe, with a white deposit in the gelatine.

No. 36 showed a white cocoon growth on the surface, on the *eighth day*, which was unchanged after *two months*.

No. 37 showed white cocoons and a small liquefying globe, with a white precipitate, on the *eighth day*, which had increased in size on the *fourteenth day*. *Two months* afterwards, the gelatine had liquefied, and the white growth had sunk to the bottom of the test-tube.

Variolous cultivations.

No. 38 showed a small white cocoon growth, without liquefaction.

Source V. Primary.

No. 39 showed a white cocoon growth on the *eighth day*, which was much larger on the *fourteenth day*. *Two months* afterwards, the growth had increased considerably, and remained pure white.

No. 40 showed a small white cocoon, without liquefaction, after *two months'* growth.

No. 41 showed a white growth on the surface, and minute white growths below it, on the *eighth day*. On the *fourteenth day*, there was a liquefying globe, with precipitate. *Two months* afterwards, the gelatine had liquefied, and there was an orange precipitate at the bottom of the test-tube.

No. 42 showed, after *two months*, a faint white growth.

No. 43 showed, on the *eighth day*, cocoons and separate small liquefying globes, which had increased and coalesced on the *fourteenth day*. *Two months* afterwards, all the gelatine had liquefied, and a yellow precipitate occupied the bottom of the test-tube.

Primary Cultivations in Agar Agar.

Primary.

No. 44 showed a cloudy white growth after *two months*.

No. 45 showed a thick white growth on the surface on the *eighth day*, which was unchanged after *two months*.

No. 46 showed no reaction.

No. 47 showed a cloudy white growth, with minute nodules scattered through it. Variolous cultivations.

Nos. 48 and 49 showed a faint white growth. Source V. Primary.

No. 50 showed, on the *eighth* and *fourteenth days*, a thick white cloudy growth, which was white, with a dull orange or brown patch on the surface, after *two months*.

No. 51 showed no reaction.

Cultivations in Serum.

No. 52 showed a white cloudy growth after *two months*.

No. 53 showed no reaction.

Source No. VI.

Case F.—W. R., male. Early vesicular stage. Admitted to Hospital Ships, Sept. 13, 1885. No visible marks of vaccination. Date of cultivation—September 14, 1885.

Primary Cultivations in Koch's Nutrient Gelatine.

Nos. 54, 55, 56, and 59 showed a faint white cocoon growth. Primary.

No. 57 showed no reaction.

In No. 58, after *two months*, the gelatine had liquefied, and the white cocoon growth had sunk to the bottom as a white precipitate.

Variolous cultivations. Source VI. Primary.

Primary Cultivations in Agar Agar.

Nos. 60, 61, and 62 showed an opaque white growth on the surface, and an indefinite cloud below it.

Nos. 63 and 64 showed no reaction.

Cultivations in Solid Blood Serum.

Nos. 65 and 66 showed a large white cloudy growth after two months.

Source No. VII.

Case G.—C. H., male; 19 years. Decline of pustular stage. Lymph taken from bullæ on wrist. Vaccinated. Admitted to Hospital Ships, September 7, 1885. Date of cultivation—September 14, 1885.

Primary Cultivations in Koch's Nutrient Gelatine.

Primary.

Nos. 67, 68, 69, and 70 showed separate minute white cocoons in the track of the wire. There was, on the *eighth day*, on the surface of the gelatine, an area of liquefaction, shaped like an inverted cone, at the bottom of which there was a white deposit. This had increased on the *fourteenth day*, and had swallowed up the remaining cocoons, becoming funnel-shaped, with a white deposit at the bottom of the liquid. After *two months* the liquefaction had increased, but so slowly that the whole of the gelatine had not become fluid. It did ultimately liquefy entirely.

Primary Cultivations in Agar Agar.

Variolous cultivations. Source VII. Primary.

Nos. 71 and 72 showed, on the *eighth day*, a white cloud in the track of the wire and a dull orange growth on the surface of the gelatine. After *two months*, the growth was stronger, but its character remained unchanged.

No. 73 showed, on the *eighth day*, a cloudy white growth, with minute nodules in it. There was a faint orange tinge on the surface. This growth was also unchanged after two months.

No. 74 showed a thick, cloudy, dull white zone on the surface, after *two months'* growth.

No. 75 showed a thick cloudy white growth, with a commencing orange tinge, on the *eighth day*, which was distinctly increased and orange-coloured after *two months'* growth.

SECTION II.

Analysis of Table of Variolous Cultivations.

Analysis of table of variolous cultivations.

An analysis of the table of cultivations of variolous lymph shows that they were successfully obtained from five out of six sources, and that the prevailing colour was white. They may be conveniently divided into three classes:—

Three classes.

1. Where no reaction or growth occurred.
2. Where growth occurred without liquefaction.
3. Where growth was accompanied by liquefaction.

Analysis of table of variolous cultivations.

The first class is of value as showing the care with which contamination of the media was prevented. The third class was excluded from present comparison with the vaccine cultivations by the occurrence of liquefaction in Koch's nutrient gelatine.

Second class compared with vaccine cultivations.

The second class may be fairly compared with vaccine cultivations, both in Koch's nutrient gelatine and agar agar. But it had been found that the most definite and easily-recognised form of artificial vaccine growth was the cocoon, in Koch's nutrient gelatine, so that the series was reduced to cultivations of variola showing this distinct mode of growth. The cultivations in serum were rejected on account of their indefinite naked-eye appearance. Those in agar agar corresponded closely with vaccine cultivations in the same nourishing medium.

Nos. 11, 12, 39.

The cultivations, numbered 11, 12, and 39, showed a large definite white cocoon growth, without liquefaction, and they were therefore selected as parallel and probably identical with vaccine cultivations of the same appearance. The histological investigation of this white variola, and the definite results obtained when it was inoculated experimentally, confirmed this opinion. To all appearance the primary cultivation was perfectly pure, without mixture with any other organism, and pure cultivations showed no change in colour. Its action was found to be quite different from that of variolous lymph.

Histological investigation and inoculation.

Parallelism.

The parallelism between cultivations of variola and vaccinia was further established by the study of the

third class of cultivations. It was found that, where liquefaction of Koch's gelatine had taken place, the colour of the precipitate was generally white, but the liquefaction usually took place so early that no change was observed in the colour. Analysis of table of variolous cultivations.

Nos. 35, 37, and 38 are examples of liquefaction with a white precipitate. No. 41, however, which was originally a white growth, produced an orange precipitate, and No. 43 produced a yellow precipitate. No attempt was made to secure a pure orange and yellow variola, as the liquefaction was understood to show contamination. All the pure white cultivations of variola were obtained from the vesicles, at a very early stage of their progress, when the lymph was clear. Sources of colours.

The white cultivations, Nos. 67, 68, 69, and 70, from the lymph in the bullæ, were spoiled by liquefaction of the gelatine occurring; but they are very interesting, as it seems to be probable that these secondary bullæ are produced by re-absorption of the smallpox virus, from the pustules, during the decline of the disease. If so, then it appears as if the skin may be affected by the poison a second time, the local effects being manifested differently. Probable cause of bullæ.

CHAPTER V.

THE DEMONSTRATION OF MICRO-ORGANISMS IN LYMPH AND ITS CULTIVATIONS.

SECTION I.

Present State of Knowledge regarding the Nature of the Vaccine and Variolous Virus or Contagium.

Present state of knowledge.

Fifteen years ago the essential nature and composition of vaccine lymph were unknown. "True vaccine matter," or "lymph from a characteristic vaccine vesicle," is "quite clear" or "very nearly limpid." "Admixtures of imperceptible minuteness can be imagined," which are not dangerous unless visible (Simon). "It is *sui generis* altogether; it has no microscopic or chemical character, but I should judge it by its effects, just as I should judge that two seeds were different, although I could not examine them, one producing an oak and the other an elm"[1]

Simon.

Sir W. Jenner.

[1] *Report of the Select Committee of the House of Commons on the Vaccination Act*, 1867, 1871.

(Sir W. Jenner). Keber, however, before the expression of these opinions, had, in 1868, investigated the microscopic constituents of vaccine lymph, in which he found bacteria. He filtered it through paper, and inoculated both the residue and the filtrate. The former produced pustules when inoculated, while no result was produced by the latter. Chauveau and Burdon Sanderson have obtained similar results. Weigert, in 1871, described bacteria of the smallest form, which he found in the vesicles, and tissues surrounding them, of persons who had died of smallpox. Cohn, in 1872, made a most exhaustive and exact research regarding the development of organisms in both vaccine and variolous lymph. He demonstrated the presence of minute spheroidal corpuscles in fresh vaccine and variolous lymph, and, by continuous observation, made out their gradual increase in size and number, and he describes sarcina-forms, and bodies like oil drops, in stored lymph. Klebs describes this sarcina-form, or regular quadripartite arrangement, as a peculiarity of the micrococci of variola and vaccinia. In common with other micrococci, they resist the action of acids and alkalies. Klein, in 1874, also demonstrated micrococci and mycelium in sheep-pox; but, in 1876, he acknowledged that the appearance of mycelium was due to the method of preparation of the specimens. Pohl-Pincus, in 1882, examined fresh lymph taken from the pock of the calf (from the fifth to the sixth day after vaccination), and found epithelial cells, white and also red

Present state of knowledge.
Keber.
Chauveau.
Burdon Sanderson.
Weigert.
Cohn.
Klebs.
Klein.
Pohl-Pincus.

Present state of knowledge.

blood-corpuscles and micrococci. Other forms, described by Bohn, Gruenhager, he considers as not essential constituents, as he has not found them. The micrococci in fresh lymph are almost always isolated. He has not seen balls of micrococci in fresh lymph. He has not found active lymph free of micrococci. The diameter of a single micrococcus measures about 1 μ. The micrococci are introduced along with the lymph, and their number increases in the tissue of the inoculated animal.

Buist.

The author (*Proc. Roy. Soc. Edin.*, 1886) has attempted to show that clear vaccine and variolous lymph contain not micrococci but the spores of micrococci; that opaque vaccine and variolous lymph are natural cultivations of the spores contained in clear lymph; and he has attempted to prove, by experimental vaccination, that these materials and cultivations of them outside the animal body differ in potency. Clear lymph was alkaline, while opaque lymph was acid in reaction. He considers also that the organisms found in cultivations of variola and vaccinia are developmental conditions of the spores in fresh lymph.

Pfeifer.

Pfeifer[1] found always, in calf lymph, certain torula forms ("Sprosspilzformen"), which he considers to be not true yeast forms, and which, he thinks, are derived from the dust in the cowstalls. He has not found them in human lymph. The author has found these in opaque vaccine and variolous lymph, and in liquefied cultivations of both in Koch's nutrient

[1] Baumgarten, *Jahresbericht*, 1886, p. 146.

gelatine. He considers them to be probably resting-stages of the organisms in fresh lymph. Similar peculiar bodies (Eigenthümliche Körper) have been found by Pohl-Pincus in the tissues of the calf, whose significance neither he, nor skilled pathologists whom he consulted, have been able to explain to their satisfaction. He, however suggests, among other hypotheses, that they are resting-spores.

Present state of knowledge.

Pohl-Pincus.

SECTION II.

Demonstration of Micro-Organisms in Vaccine and Variolous Lymph.

Koch ("Zur Untersuchung v. Pathogenen Organismen," Cohn's *Beiträge*, Bd. ii.) has well described the difficulties attending the demonstration of bacteria in animal fluids. These difficulties are much increased when the blood, pus, lymph, or sputum contains a large amount of albumen, and when the organisms are very minute and of indefinite form. His remarks upon this subject apply in a very especial manner to the investigation of fresh vaccine and variolous lymph. Both kinds of lymph contain albumen in large quantity; the organisms they contain are very minute; and, further, they stain with difficulty. Cultivations of such lymph, on the other hand, are stained with the greatest ease. I have been taught, by painful experience and repeated disappointment, the result of neglecting Dr Koch's simple instructions as to the

Demonstration of micro-organisms in lymph.

Difficulties.

Demonstration of micro-organisms in lymph.

avoidance of watery solutions of the dyes used for staining such fluids. In the first place, drying the lymph on the cover-glass is not sufficient, it requires to be fixed, either by heat or by hardening in absolute alcohol. The albumen is thus rendered insoluble, no precipitate is formed with the dye, and decolorisation can be effectively carried out. Simple staining with aniline dyes for a few minutes, and then washing away the excess of stain by His's method, are very unsatisfactory. The organisms require a long time to stain, some hours, unless the stain is warmed, when a shorter time is sufficient. The staining solutions should be alcoholic. The lymph should be taken directly from typical vaccine vesicles, without areola, and dried on cover-glasses immediately. A comparative trial of different stains leads me to prefer, for fresh lymph, the aniline methyl-violet employed after the Ehrlich-Koch method. The difficulty experienced in staining vaccine and variolous lymph is the same as that found in staining tubercle-bacilli in sputum, and is easily overcome in the same way. It is unnecessary to decolorise the preparations by dilute nitric acid, washing in water and alcohol being sufficient. In cases where there is a doubtful or indefinite knowledge of the size and form of very minute organisms, it is advisable to confine ourselves to a single staining fluid. For demonstration of these organisms, therefore, in fresh lymph, I prefer the aniline methyl-violet or fuchsin.

Ehrlich-Koch method best.

A summary of the method is as follows:— Demonstration of micro-organisms in lymph.

1. Cover-glass preparations are made directly from typical vaccine vesicles, and kept till required, after being passed three times through the flame of a Bunsen.
2. Stain for twelve hours in freshly prepared Ehrlich-Koch solution of aniline methyl-violet.
3. If quick staining is required, the solution must be warmed. Half an hour to an hour is then sufficient.
4. Wash in distilled water. Summary of method of staining.
5. Then in 60 per cent. alcohol.
6. Dehydrate in absolute alcohol.
7. Mount immediately in balsam and benzol.
8. If desired, the dried preparation may be labelled and kept unmounted, or it may be examined merely in water.
9. Contrast staining is usually unnecessary, but, if desired, a solution of vesuvin or chysoidin may be used after washing in 60 per cent. alcohol. Dehydrate in absolute alcohol, and mount.

This method is also sufficient for the examination of lymph which has been stored in tubes for a short time only.

When the lymph has been kept for several months it is well to examine it without staining, by which means large globular oil-drop-looking bodies are easily demonstrated. These bodies stain easily with the

Demonstration of micro-organisms in lymph.

methyl-violet, but are very delicate, and preparations containing them should not be heated. It is best simply to dry them, and use the stain very much diluted. When the preparation is made by simply mixing a drop of the diluted stain with the undried lymph, the globules can be seen crowding the field and moving in the direction of the currents. When these preparations are allowed to dry, the air, as it enters below the cover-glass, collects them into groups, which lose all structure when the drying is complete. Deeply stained apparently budding masses are thus produced.

SECTION III.

Demonstration of Micro-Organisms in Vaccine and Variolous Cultivations.

In cultivations of lymph.

Cultivations in solid media, which have not undergone liquefaction, are extremely viscid, and are most easily spread by a needle. If the usual method of pressing a small portion of the cultivation between two cover-glasses be adopted, this should be done rapidly and very gently, to avoid drying and gluing of the two surfaces together, as the organisms become rolled together into masses in the act of separating the covers. It is best to mop, as it were, one of the cover-glasses with the cultivation to be examined. In this way the thinnest films can easily be obtained, and the units are best displayed. The cover-glass is then dried, passed through the flame three times, and stained with

Demonstration of micro-organisms in cultivations.

methyl or gentian violet. The excess of stain is removed by simple washing with distilled water by His's method, or Gram's method, by washing in alcohol, and then in solution of iodine and iodide of potassium, and dehydrating with absolute alcohol. The difficulty consists chiefly in spreading the film smoothly, owing to its viscidity, as may be seen by examining carefully different parts of the same preparations. Gentian violet employed after Gram's method, is perfectly suitable for the purpose, and I have used it uniformly throughout this investigation. There is a certain convenience in always using the same stain, as different preparations are thus more easily compared.

CHAPTER VI.

HISTOLOGY OF LYMPH AND ITS CULTIVATIONS.

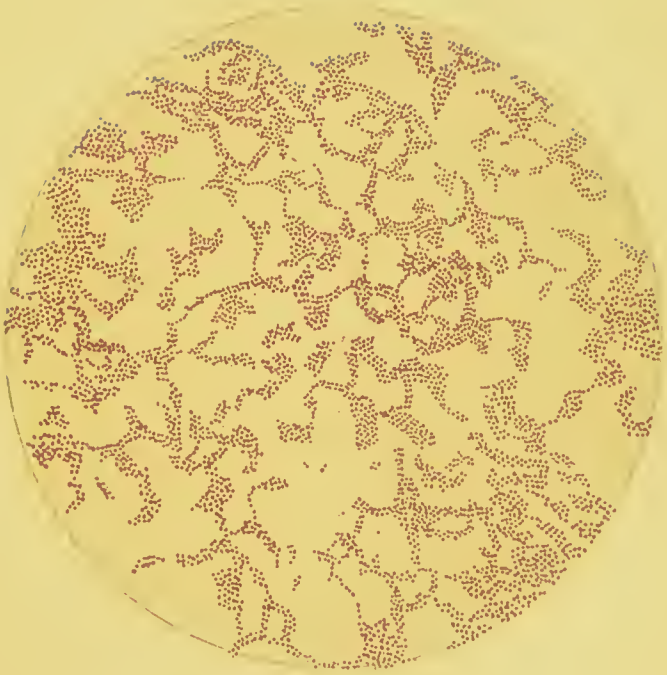
SECTION I.

1. Primary Orange Vaccine Cultivation.

Orange vaccine.

This variety is best seen in Koch's nutrient gelatine, where it forms a definite eircumseribed mass on the surfaee, and yellow or white eoeoons below the surface. The part of the growth which is not exposed to the air is pale yellow, the orange colour is superfieial. A small portion of the growth was taken from its centre, and the most superficial layer of it. It was spread on a cover-glass, dried, passed three times through the flame, and stained for a few minutes with solution of gentian violet. The exeess of stain was removed by washing in aleohol, and then in iodine and iodide of potassium solution. The preparation was dehydrated in absolute alcohol, and mounted, after drying, in balsam and benzol.

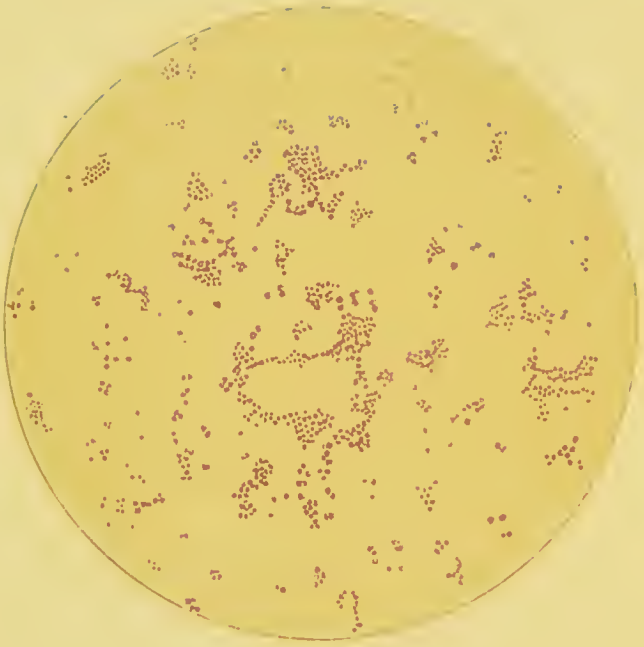
The preparation shows swarms of microeoeei, with-



PRIMARY ORANGE VACCINE CULTIVATION.

SOURCE, No. 1. Koch's nutrient gelatine. Swarms of micrococci. Size of units, .3 μ to .5 μ. Gram's gentian violet stain.

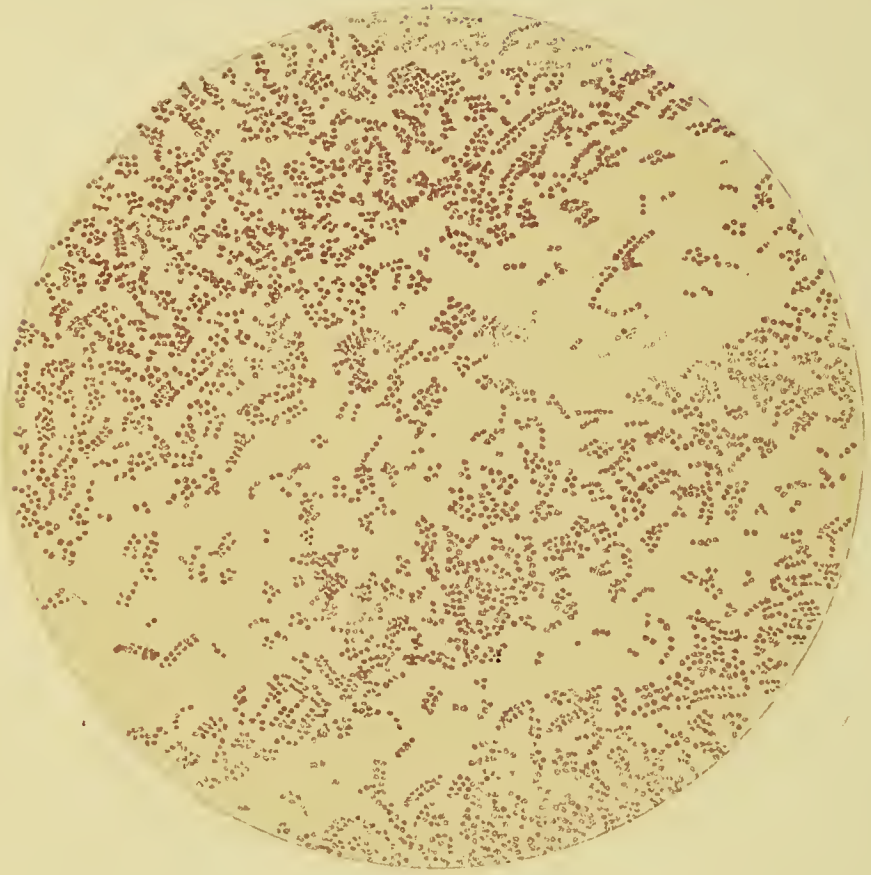
Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



PRIMARY BROWN VACCINE CULTIVATION.

SOURCE, No. 7. Agar agar gelatine. Single and double micrococci, forming sets of four, chains, and larger groups. Size of units varies from .4 μ. to 1 μ. Gentian violet.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



PRIMARY WHITE VACCINE CULTIVATION.

SOURCE, No. 7. Koch's nutrient gelatine. The preparation shews diplococci arranged in chains. The size of the units varies from .3 μ to 1 μ. Gentian violet.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.

SCOTT & FERGUSON, EDIN.

out definite arrangement in chains or tetrads. The size of the units, measured by Zeiss, Objective K, Water immersion, Eyepiece micrometer No. 2, is ·3 μ to 5 μ. The chromo-lithograph was drawn by camera under a power of 1045. Zeiss, obj. K., oc. 3. Orange vaccine.

2. Primary Brown Vaccine Cultivation.

This was found in cultivations in agar agar, and corresponds apparently to the orange cultivation in Koch's nutrient gelatine. The growth is not so distinctly defined, and the difference in colour may depend on the different culture medium. Brown vaccine.

The preparation shows swarms of micrococci, without definite arrangement, exactly like those seen in orange vaccine.

The size measured by Zeiss, obj. K., oc. mic. No. 2, is ·4 to ·8 μ to 1 μ. The larger organisms also represent units. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Magnification, 1045. Stain, gentian violet.

3. Primary White Vaccine Cultivation.

The preparation was made from a pure white growth in Koch's nutrient gelatine. It shows single and dumb-bell micrococci, with a few sets of four, here and there. The micrococci are almost universally arranged in chains of various lengths. White vaccine.

The size measured by Zeiss, obj. K., oc. mic. No. 2, varies from ·3 μ to ·1 μ in diameter. The camera drawing was made with Zeiss, obj. K., oc. No. 3. Magnification, 1045. Stain, gentian violet.

4. Yellow Vaccine Cultivation.

Yellow vaccine.

This preparation was taken from a pure growth in agar agar, and shows simple and compound sarcina-forms. Different parts of the preparation show the developmental stages:—(*a*) Primitive sarcina-forms; (*b*) larger simple tetrads; (*c*) compound sarcina-forms.

The units, measured by Zeiss, obj. K., oc. mic. No. 2, are ·1, ·2, ·4, ·5 μ to 1 μ in diameter. The sarcinæ vary in size, being from ·5 μ to 2 μ in diameter. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Magnification, 1045. Stain, gentian violet.

5. Yellow Cocoon from Orange Cultivation.

Yellow cocoon.

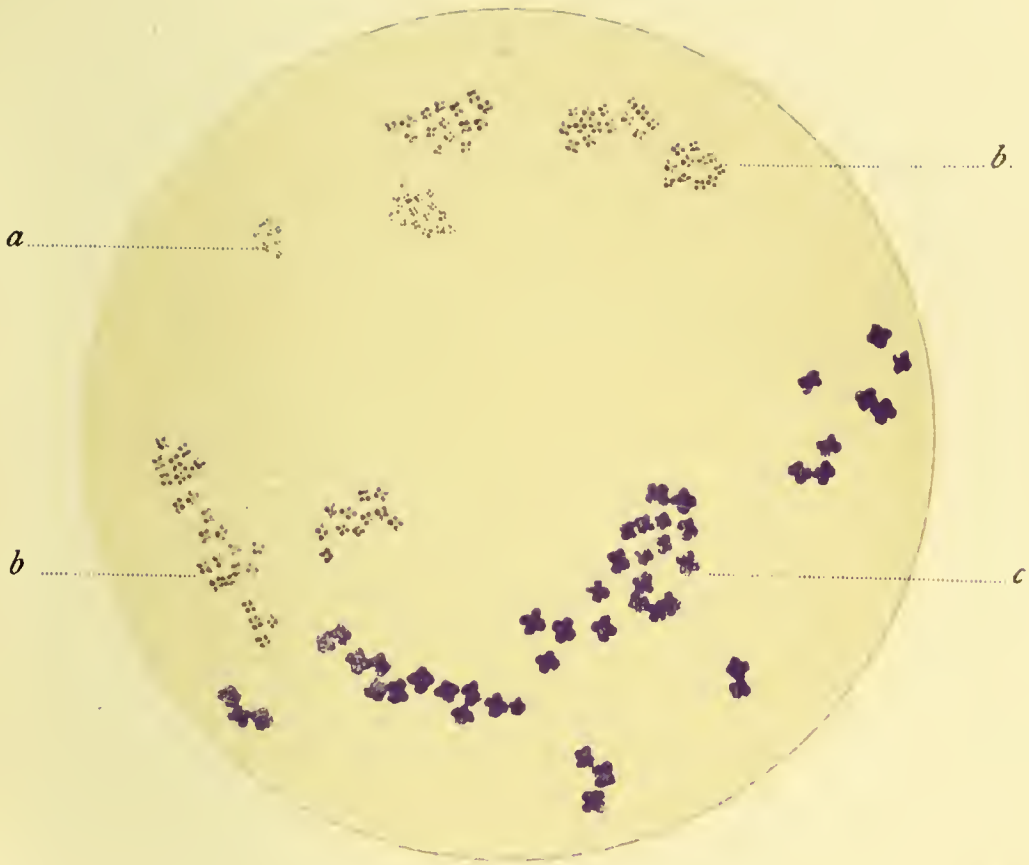
This preparation was taken from a yellow cocoon below the surface of the gelatine. The left half of the drawing shows small lozenge-shaped sarcinæ, or sets of four, and the right half larger sarcinæ from another part of the slide, the units of both being apparently simple. The size of the units measured by Zeiss, obj. K., oc. mic. No. 2, is ·4 μ on the left, and ·8 μ on the right. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Magnification, 1045. Stain, gentian violet.

6. White Cocoon from Orange Cultivation.

White cocoon.

This preparation was made from a white cocoon growing beneath the surface of the gelatine. It shows simple tetrads made up of very minute units. The size of the units, measured by Zeiss, obj. K., oc. mic.

PLATE IV.



YELLOW VACCINE, PURE CULTIVATION.

SOURCE, No. 2. Agar agar gelatine.

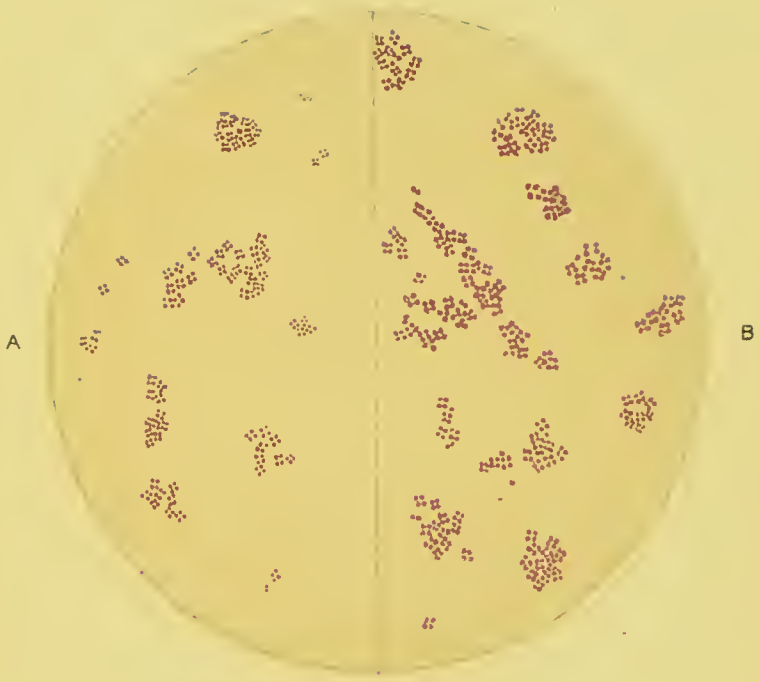
a Primitive sarcina-forms. *b b* Larger primitive tetrades.

c Compound sarcina-forms.

Size of elementary units varies from .2 μ to .4 μ, and .5 μ to 1 μ. Size of sarcinae, varies from .5 μ to 2 μ. The sets of four are lozenge-shaped, as if composed of diplococci. Gentian violet.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.

SCOTT & FERGUSON EDIN.



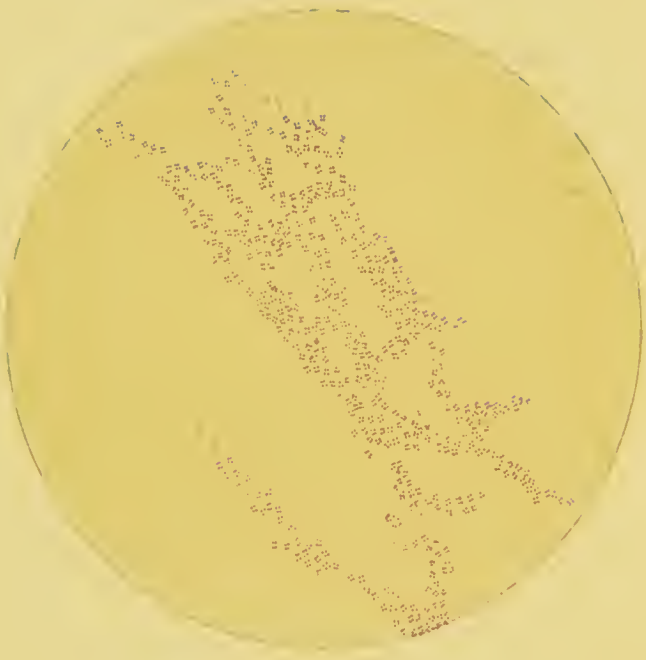
YELLOW COCOON FROM PRIMARY ORANGE VACCINE CULTIVATION.

SOURCE, NO. 1. Koch's nutrient gelatine.

a (Left half). Diplococci arranged like sarcinae. Size of units, .4 μ. The sets of four are lozenge-shaped.

b (Right half). Larger diplococci, arranged like sarcinae. Size of units, .8 μ. The sets of four are lozenge-shaped. Gentian violet stain.

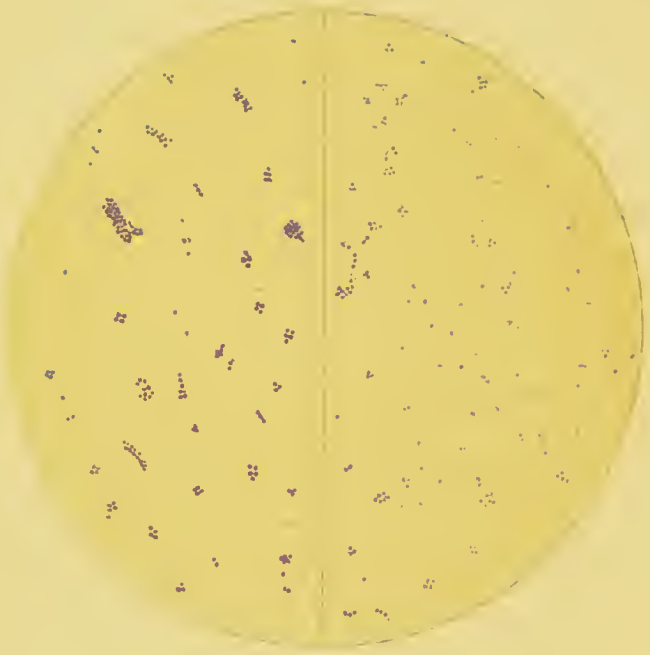
Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



WHITE COCOON FROM PRIMARY ORANGE VACCINE CULTIVATION.

SOURCE, No. 1. Koch's nutrient gelatine. Very minute diplococci arranged like sarcinae. Size of units, .1 μ to .2 μ. The sets of four are lozenge-shaped. Gentian violet stain.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



ORANGE VACCINE, PURE CULTIVATION.

a (Left half).

SOURCE, No. 1. Koch's nutrient gelatine. Single micrococci and diplococci. The larger groups and chains are composed of these. Size of units, .5 μ. Gentian violet.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.

WHITE VACCINE, PURE CULTIVATION.

b (Right half.)

SOURCE, No. 7. Koch's nutrient gelatine. Single and dumb-bell micrococci. The groups and chains are composed of these placed close together. Size of units, .5 μ.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.

No. 2, is ·1 to ·2 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, gentian violet. White cocoon.

7. Orange Vaccine, Pure Cultivation.

Plate VII.(A.) Left half shows, in Koch's nutrient gelatine, single micrococci and diplococci. The preparation also shows groups, which contain three, four, or more micrococci. Where four organisms are seen together, they assume a lozenge shape, different from the true tetrad. Those look like two pairs of dumb-bells placed side by side. Short chains are also seen. The larger groups and chains are probably made up of diplococci. The ground substance appears faintly stained between the individual organisms. Pure orange vaccine.

The size of the units, measured by Zeiss, obj. K., oc. mic. No. 2, is ·5 μ. The camera drawing was made with Zeiss, obj. K., oc. 3. Magnification, 1045. Stain, gentian violet.

8. White Vaccine, Pure Cultivation.

Plate VII. (B.) Right half, shows single and dumb-bell micrococci. The groups and chains of three and four are composed of these placed close together. They are indistinguishable in size and arrangement from orange, pure cultivation. The size of the units, measured Zeiss, obj. K., oc. mic. No. 2, is ·5 μ in diameter. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, gentian violet. Pure white vaccine.

9. Clear Vaccine Lymph.

Clear vaccine lymph.

This cover-glass preparation was taken directly from a typical vaccine vesicle, and dried immediately. It shows minute spherical spore-like micrococci, mostly isolated. A few pairs of organisms are seen. The size measured by Zeiss, obj. K., oc. mic. No. 2, varies from ·1 μ to ·5 μ. Stain, aniline methyl-violet. The camera drawing was made by Zeiss, obj. K., oc. No. 3.

10. Opaque Vaccine Lymph.

Opaque vaccine lymph.

This preparation was made by Dr Francis Troup from vaccine lymph stored for some time in an ordinary commercial vaccine tube.

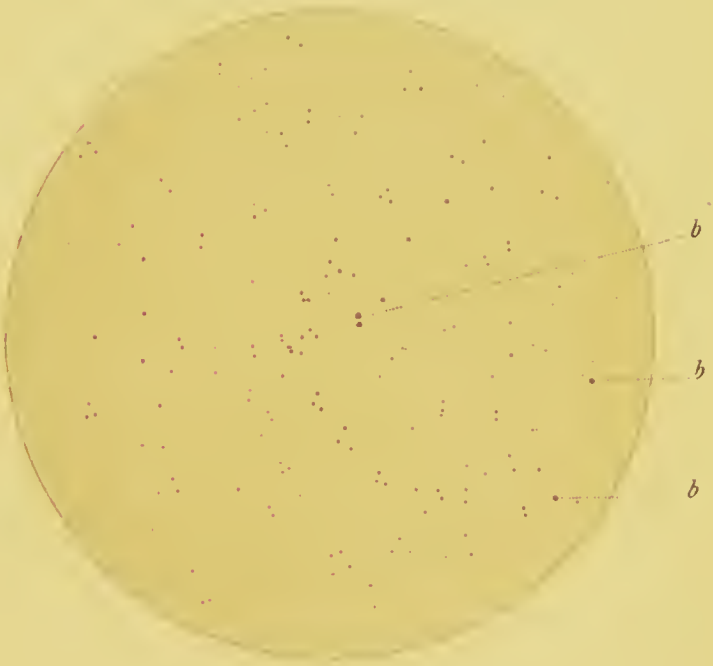
It shows—(*a*) chains of micrococci; (*b*) diplococci and tetrads; (*c*) large single torula-looking bodies; (*d*) large deeply stained masses showing swarms and chains of micrococci; (*e*) ground staining of film.

The size of the micrococci is 1 μ and that of the torulæ 3 μ, measured by Zeiss, obj. K., oc. mic. No. 2. The camera drawing was made by Zeiss, obj. K., oc. 3. Magnification, 1045. Stain, aniline methyl-violet.

11. White Variola, Pure Cultivation.

Pure white variola.

This preparation was taken from a pure cultivation of white variola in Koch's nutrient gelatine. It shows almost universally dumb-bell micrococci arranged in various ways. There are a few tetrads, probably not true. The size of the units measured by Zeiss, obj. K., oc. mic. No. 2, varies m ·4 μ to ·8 μ. The camera draw-



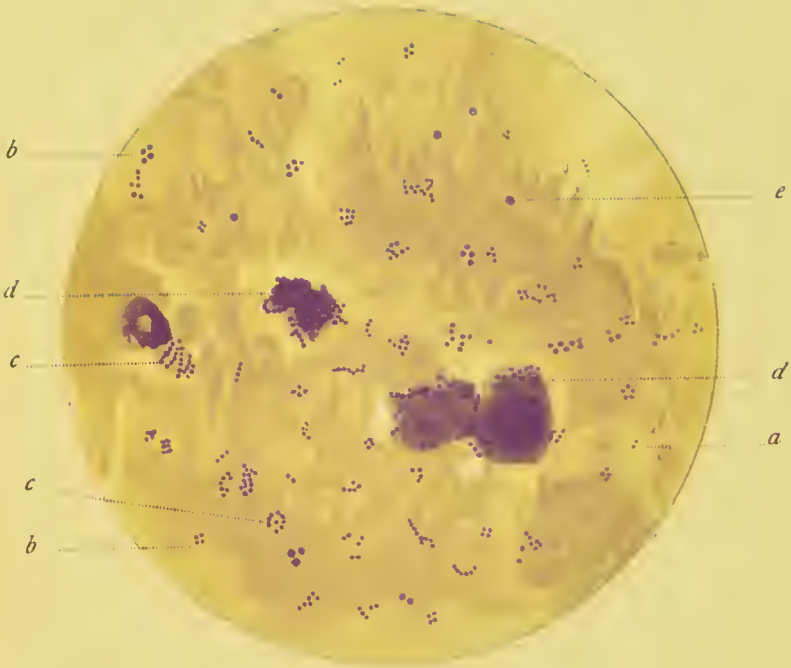
CLEAR VACCINE LYMPH.

SOURCE, Typical Jennerian vesicle. Cover-glass preparation.

a Minute isolated spores of micrococci.

b b b Larger forms of micrococci, showing commencement of opacity. Size of units varies from .1 μ to .5 μ. Koch's aniline methyl-violet stain.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



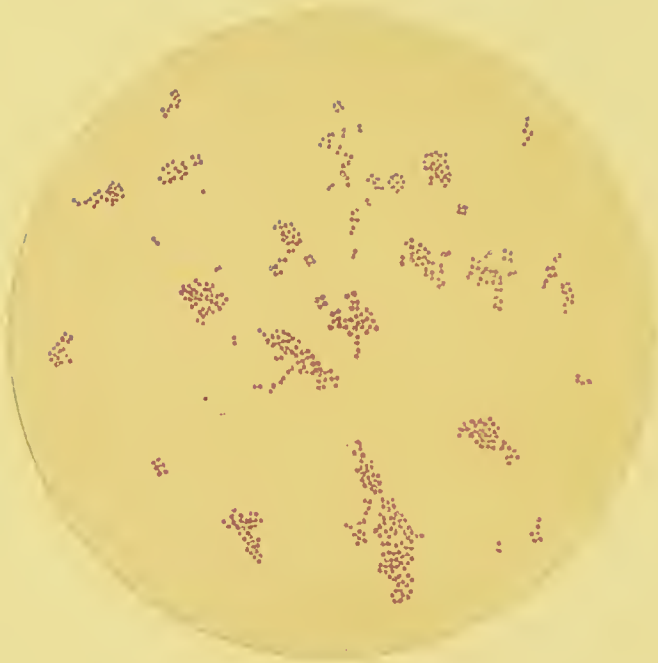
OPAQUE VACCINE LYMPH.

SOURCE, Preparation by Dr Francis Troup, *from lymph in capillary tube.* This preparation shows :—*a* Diplococci. *b b* Sarcina-form groups. *c c* Chains or necklaces. *d* Irregular colonies or clumps. *e* Very large torula-form micrococci, like oil-drops when unstained. The size of the organisms varies from .5 μ to 2 μ. Koch's aniline methyl-violet stain.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.

SCOTT & FERGUSON, EDINT.

PLATE X.

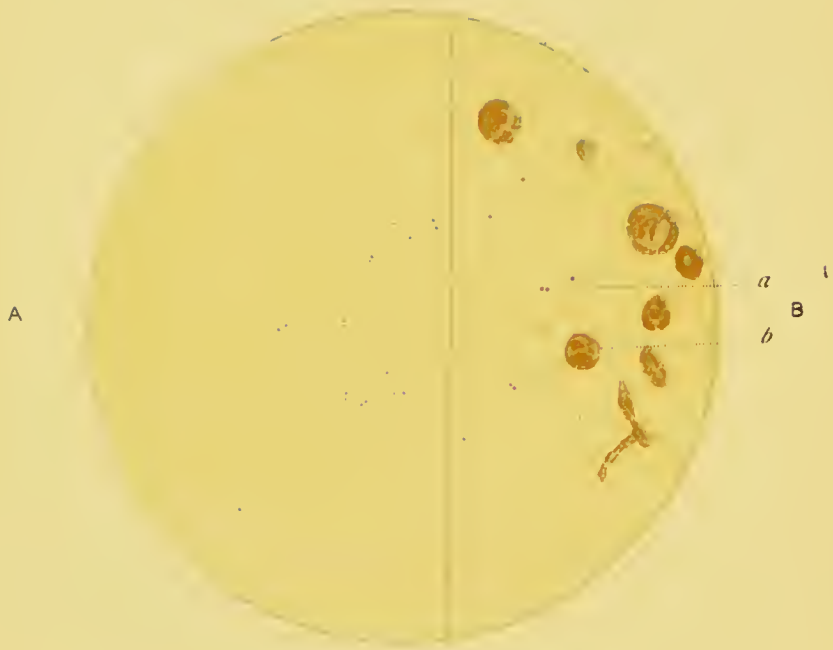


WHITE VARIOLA. PURE CULTIVATION.

SOURCE, No. 2. *Cultivation*, *No.* 12. Koch's nutrient gelatine. Dumb-bell micrococci, arranged in various ways. There are a few lozenge-shaped sets of four, not true tetrades.

Size of units, varies from .4 μ to .8 μ. Gentian violet stain.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



CLEAR VARIOLOUS LYMPH.

a (Left half).

SOURCE, *Cover-glass preparation from early vesicle* of a case of variola in the Hospital Ships, Purfleet. Shows spores of micrococci. Size, .2 μ to .3 μ. Gentian violet.

Zeiss, Obj. K, Oc. 3. × 1045.

OPAQUE VARIOLOUS LYMPH.

b (Right half.)

SOURCE, *Cover-glass preparation from contents of pustule* of a case of variola in the Hospital Ships, Purfleet. Shows—*a* larger micrococci and diplococci. *b* Torulæ variolæ. Size of micrococci, .5 μ; of torulae, 3 μ to 8 μ. Gentian violet and chrysoidin.

Zeiss, Obj. K, Oc. 3, × 1045.

ing was made by Zeiss, obj. K., oc. No. 3. Stain gentian violet. Pure white variola.

12. (A.) Clear Variolous Lymph.

The left half of the drawing was made from a cover-glass preparation taken directly from a variolous vesicle of a patient in the Hospital Ships, Purfleet. It shows very minute spore-like micrococci embedded in the film:—(*a*) isolated spores; (*b*) pairs of organisms. The size measured by Zeiss, obj. K., oc. mic. No. 2, is ·1 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain gentian violet. Clear variolous lymph.

(B.) Pustular Variolous Lymph.

The right half of the drawing was made from a cover-glass preparation taken from a variolous pustule of a patient in the Hospital Ships, Purfleet. It shows—(*a*) single and dumb-bell micrococci stained with gentian violet; (*b*) large torula-looking bodies stained brown by chrysoidin; (?) pus cells; (*c*) altered cell. Pustular variolous lymph.

The size of the micrococci measured by Zeiss, obj. K., oc. mic. No. 2, is 1 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stains, gentian violet and chrysoidin.

13. (A.) Torulæ Variolæ.

The left half of the drawing shows—(*a*) numerous torulæ of various sizes, 4 to 8 μ; (*b*) deeply stained burst cell. The size was measured by Zeiss, obj. K., oc. mic. Torulæ variolæ.

Torulæ variolæ

No. 2. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, aniline methyl-violet.

(B.) Torulæ Vaccinæ.

Torulæ vaccinæ.

The right half of the drawing shows perfect torulæ; others which have burst; and isolated micrococci. The preparation from which the drawing was taken had been kept a considerable time. (*a*) Perfect torulæ; (*b*) pale irregular bodies which are the remains of spherical cells; (*c*) deeply stained micrococci. The size of the torulæ measured by Zeiss, obj. K., oc. mic. No. 2, varied from 2 to 3 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, gentian violet.

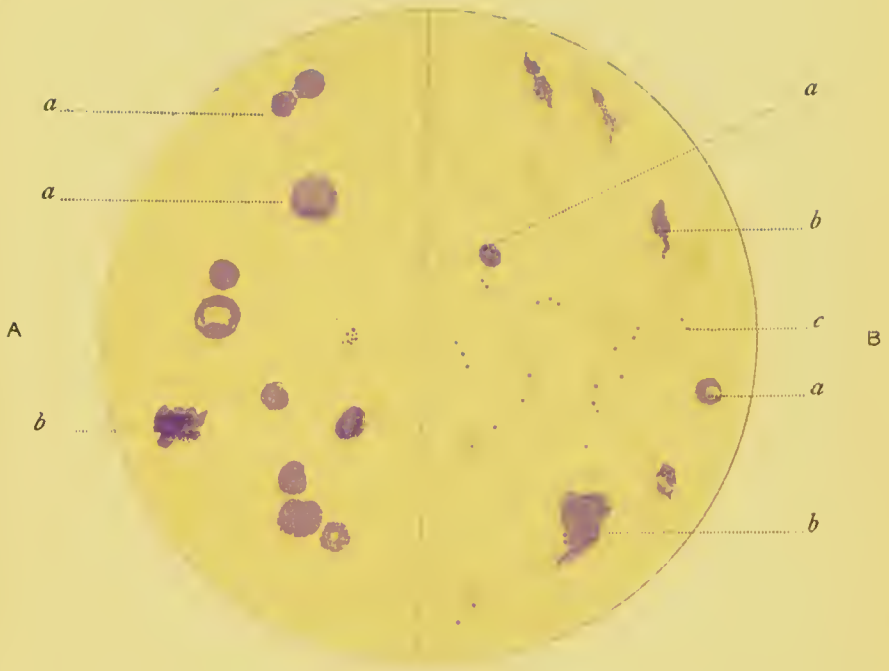
SECTION II.

1. MICROSCOPICAL APPEARANCES OF DRIED YEAST.

14. Pure Dried Alcoholic Yeast.

Dried yeast.

This preparation was unstained. It shows yeast cells containing large nuclei and nucleoli. The nucleus in many of the cells is pressing aside the protoplasm, in which numerous refractive corpuscles, like spores, can be seen. Some nuclei have single nucleoli, others have two, and in some cells two nuclei with nucleoli are seen. The size of the cells measured by Zeiss, obj. K., oc. mic. No. 2, varies from 3 μ to 8 μ.



TORULÆ VARIOLÆ.

a (Left half.)

SOURCE, *Cultivation of variola, No.* 41, *orange precipitate.* Fresh preparation.

a a Spherical cells. *b* Irregular mass, probably originally spherical. Size, 2 μ to 5 μ. Gentian violet.

Zeiss, Obj. K, Oc. 3. × 1045.

TORULÆ VACCINÆ.

b (Right half.)

SOURCE, Opaque vaccine lymph. Old preparation.

a a Spherical cells. *b b* Irregular masses, originally spherical.

c Micrococci. Gentian violet. Size, 2 to 3 μ.

Zeiss, Obj. K. Water immersion. Oc. 3. × 1045.

PURE DRIED ALCOHOLIC YEAST.

SOURCE, *Fermenting alcoholic wort.* Unstained preparation, showing endogenous cell-formation.

a Small cell containing nucleus and bright refractive bodies in the protoplasm.

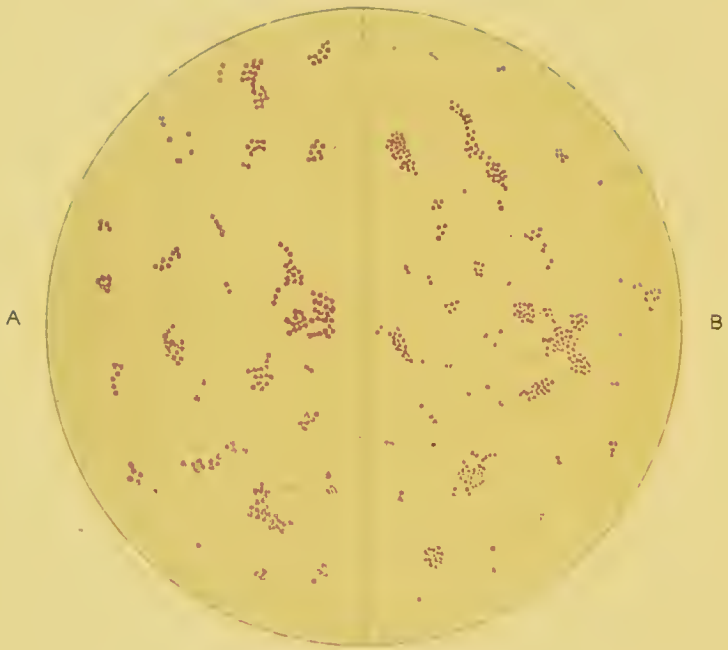
b Larger cell with nucleus and nucleolus.

c c c Cells with a single oval nucleus containing two nucleoli.

d Cell with two nuclei containing single nucleoli.

The size of the cells varies from 3 μ to 8 μ.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters.



MICROCOCCI FROM YEAST SCAB.

a (Left half).

SOURCE, *Yellow cultivation from blood of monkey after fermentation with dried yeast.* Koch's nutrient gelatine. Single micrococci and diplococci, arranged in chains and groups. Size of units, 1 μ.

b (Right half).

SOURCE, *White cultivation from blood of monkey after fermentation with dried yeast.* Koch's nutrient gelatine. Single and dumb-bell micrococci in chains and swarms. Size of units, varies from .5 μ to 1 μ.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification, 1045 diameters. Gentian violet.

2. MICROSCOPICAL APPEARANCES OF CULTIVATIONS OF FERMENTED BLOOD.

15. (A.) Yellow Cultivation from Blood of Monkey after Fermentation with Dried Yeast.

The left half of the drawing shows single, dumb-bell, and chains of micrococci. The size measured by Zeiss, obj. K., oc. mic. No. 2, is 1 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, gentian violet. Micrococci from yeast.

(B.) White Cultivation from Blood of Monkey after Fermentation with Dried Yeast.

The right half of the drawing shows single, dumb-bell, and chain micrococci, and also swarms. The micrococci are smaller than those from the yellow growth. The size measured by Zeiss, obj. K., oc. mic. No. 2, varies from ·5 μ to 1 μ. The camera drawing was made by Zeiss, obj. K., oc. No. 3. Stain, gentian violet. Micrococci from yeast.

CHAPTER VII.

PURE CULTIVATIONS OF VACCINIA AND VARIOLA.

Cause of vaccinia and variola.

In order to establish the assertion that bacteria are the cause of variola and vaccinia, it is absolutely necessary to prove—

Proof necessary.

1. That bacteria are present in these diseases without exception.
2. That they exist in sufficient number in both, to account for the symptoms.
3. That they possess a well-marked and easily recognisable form.
4. That a specific significance can be attributed to this form of bacterium.

It has been proved by various able investigators that micro-organisms are present in great numbers in the contents of the vesicles of both variola and vaccinia; and that they possess a specific significance is proved by the fact that they have a definite physiological action when inoculated. But the chief difficulty in accepting the view that these micro-organisms cause variola and vaccinia, lies in the fact that they are so

Difficult.

minute that their form is neither well-marked nor easily recognisable; and that bacteria of the same form and minuteness occur in other vesicular and pustular diseases of the skin. Why proof is difficult.

Koch (*loc. cit.*) maintains that "a distinct bacteric form corresponds to each disease, and this form always remains the same, however often the disease is transmitted from one to another." In estimating, therefore, the differences between such minute bacteria, "which border on the invisible," he takes into account the conditions of their growth and their distinctly characterised physiological action, and provisionally regards different forms of bacteria, *e.g.*, spherical and rod-shaped, as different species. He considers that pure cultivations of very minute bacteria in apparatus are subject to unavoidable sources of fallacy, owing to the absence of distinct morphological characters. Failing these, however, he advocates the tracing of the whole history of the development of the bacterium from spore to spore regarded by Brefeld as necessary to justify the formation of distinct species. His opinion is, that pure cultivations of pathogenic bacteria are best carried out in the animal body, which may be regarded as completely isolated, with respect to other forms of bacteria than those intentionally introduced. Koch regards the successive transmission of artificial infective diseases as the best and surest method of pure cultivation. It can further claim the same power of demonstrating the existence of specific forms Opinions of Koch as to formation of species of bacteria. Opinion of Brefeld. Koch's method of pure cultivation.

Pure cultivations.

of bacteria, as must be conceded to any faultless cultivation experiments.

Variola.

Inoculation for smallpox may be considered as a pure cultivation of the variolous contagium, but it does not appear, as a rule, to have been transmitted successively from one person to another, the virus being always derived *de novo* from a case of variola.

Vaccinia.

Successful vaccination at present depends on due selection of subjects, choice of proper material, and method of operating, and it may be regarded as the pure cultivation of the vaccine contagium obtained empirically. A skilful vaccinator, under favourable conditions, has no great difficulty in obtaining uniform results in this empirical manner, and these must be regarded as pure cultivations of the contagium in the animal body. If, now, the bacteriologist uses the same material for starting artificial cultivations outside the animal body, the growths obtained should be recognisable by definite characters.

Artificial media.

Each artificial cultivation represents a vaccination of inoculation, and the results obtained should not differ, more than one vaccine or variolous vesicle differs from another. Definite growths of this character can only be obtained in solid media, and these must be carefully studied. The first thing to be done is to ascertain whether lymph grows in the medium, and then the mode of growth must be carefully described. As a matter of fact, we find that lymph may be grown in various media, so that a selection has to be made from

these of the medium in which the lymph-growth is most definite. Pure cultivations in artificial media.

Careful vaccination and variolation of different nourishing materials, and observation of the growths from day to day, lead me to the conclusion that Koch's nutrient gelatine is the most convenient for the artificial cultivation of variola and vaccinia. Characteristic appearances are seen in it, such as growths of various colours, white, yellow, and orange, which do not liquefy the gelatine. The circumscribed cocoon form of growth is a valuable diagnostic. Diagnosis. The colour of the surface growth is the most important, and when these are examined, we find that some vaccine vesicles produce a white growth, others a yellow, and others again an orange growth. That is to say, that lymph cultivated outside the animal body, in Koch's nutrient gelatine, produces white, yellow, or orange vaccination, according to the source from which it is derived. These varieties retain their characters in pure cultivations, the only modification being a certain loss of brightness in the pure orange cultivations.

That the results obtained cannot be regarded as due to accidental impurities, is proved by the remarkable agreement as to these among skilled bacteriologists. Results difficult to explain. There is, however, by no means the same unanimity among bacteriologists as to the explanation of these forms of growth, and the difficulty is increased when we consider that, so far as is at present known to me, no bacteriologist has succeeded in reproducing vaccinia

Pure cultivations.

Quist's experiments.

by pure cultivations in solid media. Quist has succeeded in cultivating lymph in a fluid medium, but his cultivations are not pure, according to present ideas. His results, however, are remarkable, and it is possible that they may succeed in producing some modification of these ideas. I have not seen Quist's own explanation of his success, and I have no experience of artificial cultivations of lymph in fluids. In order to arrive at a correct explanation of the true nature of vaccine cultivations, the subject must be studied from several points of view.

Comparison of lymph and cultivations.

It is almost as easy, in my opinion, to produce an artificial cultivation of the vaccine material, as it is to produce a pure cultivation of the material in the animal body. How is it, then, that they differ? When we compare clear lymph with any of the vaccine cultivations, we are at once struck by the remarkable fact that their histological appearances are totally different. Also, opaque lymph has a very different appearance from clear lymph. It is evident that there is a distinct difference in the composition of the materials. Experimental vaccination proves that these materials differ not only in the form of the organism, but also in physiological action. The conclusion, then, is irresistible, that these bacteria are not in that form the cause of either local variola or vaccinia. But it is also manifest that, if lymph can be cultivated at all artificially, it must be genetically connected with the growths produced. It is nearly impossible to conceive

Different composition of materials.

of accidental contamination in the many thousands of inoculations of artificial media, which have doubtless been performed by skilled specialists. If they have erred, it is inconceivable that all of them have committed the same blunder. For a rational explanation of the failure to cultivate lymph, we must look to the faulty method, and not to the mistakes of investigators. While, therefore, a bacterium of constant form, to be found in clear vaccine and variolous lymph, is probably the cause of variola and vaccinia, it appears to be practically impossible to reproduce the same bacterial form by artificial cultivation of lymph in solid media outside the animal body. If this be admitted, how are the results to be explained? Each artificial cultivation of variola and vaccinia must be regarded as a vaccination. The lymph is implanted in the nourishing material, in precisely the same manner and amount as in the animal body. Under suitable conditions of temperature its active principle multiplies, producing in the one case a vaccine vesicle, and in the other a white, yellow, or orange growth, as the case may be.

Pure cultivations.

Faulty methods explain failures.

Explanation of results.

According to this view, white, yellow, and orange vaccine cultivations grow directly from the organisms in the lymph. I regard the colour of the growth as quite immaterial, and dependent probably on circumstances connected with the source from which the lymph used for the special cultivation was originally derived. This view is supported by the different

Colour of growth not material.

Pure cultivations.

appearanees which are presented by growths of vaeeine lymph, derived from the same source, in Koeh's gelatine and agar agar gelatine. The eolour of the growths in agar agar is dimmer than that of the growths in Koch's gelatine. For example, orange eolour in the one is brown in the other. This is proved, on histologieal examination, by their identical size and arrangement. Then, again, all the growths in the early stage were white, no matter what the souree or the nutrient material might be; and I therefore regard white vaceine and variolous cultivations as the most typieal. The orange and yellow are, in my opinion, mere varieties of the same organism. When we examine these varieties of vaeeine eultivations, we find very different appearanees. These vary in size and arrangement aceording to the age of the cultivation, its purity, and its mode of preparation. But however mueh these may vary, none of them can be demonstrated in clear lymph. The units are almost without exception larger, none under ·5 μ, frequently they measure 1 μ, while the units in elear vaccine and variolous lymph are only about ·15 μ. We are thus forced to conclude that the organisms in vaeeine and variolous eultivations are growth-forms or developmental eonditions of the organisms in elear lymph. I regard the organisms in clear lymph as spores, whieh develop by artificial eultivation in solid media into the larger forms. Clear vaecine and variolous lymph, therefore, eontain spores of bacteria in suspension, which are distinguished from the

Varieties of the same organism.

Growth-forms or developmental conditions.

micrococci of cultivation by their characteristic difference in size. Pure cultivations.

Much the same views must be held as to opaque lymph, which is an imperfect material for vaccination. When we examine opaque lymph microscopically, we find that there is a great increase in the number of organisms, and that they are distinctly different in size and arrangement. The units are at least ·5 μ, and often larger; they are aggregated in masses, and form also rows or chains and sarcina forms. On comparing the plates it will be seen at once that opaque lymph differs so much from clear lymph that it is impossible to mistake one material for the other. Opaque lymph.

Peculiar spherical bodies, like oil drops, are frequently almost invariably, found in lymph which has been stored for a considerable time. They vary in size from 1 μ to 2 μ to 5 μ, and their nature is difficult to make out. After a careful study of these peculiar bodies, I have come to the conclusion that they are either true yeast cells, or a species of torula. They differ from the micrococci in staining with great ease, and they are very delicate, for which reason they are difficult to preserve in mounted preparations. They thus differ from red and white blood corpuscles; and, besides, they are much smaller. When present in large numbers, they cannot be mistaken for blood corpuscles. They are also easily seen in fresh preparations, so that they cannot be produced mechanically. They also occur in opaque variolous lymph, and in cultivations of both Torulæ. Their characters.

Pure cultivations. Opaque lymph.

vaccine and variolous lymph which have undergone liquefaction. They are isolated bodies, and I can come to no other conclusion than that they develop in opaque lymph from organisms pre-existing in clear lymph.

The chemical reaction of opaque lymph appears to throw some light on the essential nature of these bodies. Clear lymph is distinctly alkaline in reaction. If, however, lymph from a vesicle with much areola be tested, as it issues from the vesicle, it will be found to have an acid reaction. Further, when opaque lymph is stored its opacity increases in proportion to the length of time it is kept. It is also acid in reaction. It is thus evident that stored lymph undergoes acid fermentation. It is quite possible, though not probable, that stored lymph is always accidentally contaminated by the same ferment, and that the acidity is produced in this way. But how are we to account for acidity in lymph issuing from a newly-opened vaccine vesicle? We can only do so by regarding the cause of the acidity as intrinsic in the lymph itself. Then acid fermentation is produced outside the body by the growth of an organism, so that we naturally conclude that acidity of lymph within the vesicle is also produced by the growth of an organism. Microscopic examination of opaque lymph shows that a great development of organisms has taken place in it, so that the acidity must be due to this.

Cause of acidity.

Cause of opacity.

Another question is, What is the cause of opacity

of lymph? When we consider that clear lymph becomes opaque within the vaccine vesicle, and that fresh lymph, clear when stored, also becomes opaque; that the opacity gradually increases in amount; and that ultimately the alkaline lymph becomes acid, we are forced to conclude that opacity of lymph is due to a natural cultivation of the organisms in clear lymph, both inside and outside the animal body. The sudden appearance of opacity within the vesicle in a fluid which we know contains germs, can only be satisfactorily explained in this way. Degeneration of the fluid or migration of the leucocytes does not explain the appearances which invariably take place, for I have not been able to find leucocytes in opaque lymph. Then the opacity gradually increases outside the body, and this can only be explained by the view that the organisms in lymph are capable of development, so long as the material is suitable for their nutrition. They are not dead in stored lymph, they are only quiescent, or in a resting stage, produced by exhaustion, for the time, of their nourishing material. When re-inoculated, a new cycle of life commences. I regard opaque lymph, then, as a pure cultivation of the spores in clear lymph. If it is desired to prevent development of the spores, the lymph must be dried. Partial prevention may be secured by storing the lymph in well-filled sterilised tubes, from which the air has been expelled before they are hermetically sealed.

Pure cultivations. Cause of opacity.

Pure cultivation of the spores in clear lymph.

The discovery of yeast forms in opaque lymph raised

Origin of the spores.

Pure cultivations.

Origin of the spores.

the question in my mind whether the organisms in vaccine and variolous lymph might not actually be the spores of a species of yeast. The observation of acidity in opaque lymph, both within and without the vesicle, strengthened this impression, and led me to make an extensive series of observations and experiments to determine whether an ordinary yeast ferment could grow in warm-blooded animals and produce disease.

CHAPTER VIII.

EXPERIMENTAL VACCINATION, VARIOLATION, AND FERMENTATION.

Experiment I.

Experimental vaccination.

Vaccinifer—Calf A. Date of Vaccination—August 27, 1885. Vaccine Material—White, yellow, and orange vaccine.

Exp. I. Calf. White, yellow, and orange vaccine.

The method of vaccination was the same as that employed by Dr Cory at the National Vaccine Establishment. The groins were first shaved quite clean, and the material was then rubbed into fifty or sixty superficial scratches half an inch long. Three test-tubes, containing white, yellow, and orange cultivations of vaccine lymph, were employed. Fifteen insertions of *white* vaccine were made on the left thigh. Twenty-five insertions of *yellow* vaccine were made on the abdomen. Twenty insertions of *orange* vaccine were made on the right thigh.

Next day the animal was quite well, but the marks looked slightly inflamed. On the *third day* they were completely healed. The calf was under daily observation for a fortnight, but no local result was produced.

Experimental vaccination. Eruptions.

Nearly *five weeks* after the operation, it was discovered, incidentally, that an eruption had appeared on the top of the animal's head, between *two* and *three weeks* before, just after my daily visits had ceased. It was being rubbed with vaseline. On examination dry scabs and thinness of the hair were noticed at the affected part.

Experiment II.

Vaccinifer—Calf B. Date of Vaccination—September 8, 1885. Vaccine Material—Opaque variolous lymph.

Exp. II. Calf. Variolous lymph.

A case of smallpox having occurred in Edinburgh, the opportunity was taken to secure some variolous lymph on the eighth day of the eruption. Mr Ceely and Mr Badcock have succeeded in producing vaccinia with variolous matter, and I desired to repeat their experiments. With the assistance of Mr Reginald Bowman, M.B., New South Wales, fifty insertions were made in the usual manner. After the operation the animal was said to "pine." Next day the scratches looked red and slightly inflamed. *Four days* after the operation, all traces of it had disappeared. *Ten days* afterwards, the animal was apparently quite well. Observation was then interrupted for nearly a week, during my visit to the Hospital Ships, Purfleet, to obtain cultivations of variola. *Three weeks* after the operation, a patch of hair on the animal's back, which looked thin, attracted my notice, and, on examination, I found the remains of an eruption. It covered an

Eruption.

area the size of the palm of the hand, and it was quite dry, having scabbed. This observation led to the examination of the other calf and the discovery of the eruption on its head. Experimental vaccination. Exp. II. Calf.

Experiment III.

Vaccinifer—Calf A. Date—September 29, 1885. Vaccine Material—Variolous lymph from Case A., Purfleet.

In order to test, if possible, the protection afforded by the previous vaccination of this calf with vaccine cultivations, it was variolated with vesicular lymph, from Case A., Purfleet. Eighteen days afterwards, no result, either local or general, had been produced. Exp. III. Calf. Nil.

Experiment IV.

Vaccinifer—Calf B. Date—September 29, 1885. Vaccine Material—Opaque variolous matter from Case C., Purfleet.

To test the protection afforded by variolation three weeks previously, Calf B was inoculated again with lymph from the Hospital Ships. No result was produced. Exp. IV. Calf. Nil.

Experiment V.

Vaccinifer — Guinea-pig. Date — October 24, 1885. Vaccine Material—Orange vaccine. Exp. V. Guinea-pig.

A small area of skin over the left loin was shaved

Experimental vaccination.

Exp. V. Guinea-pig.

Local scab.

clean, and a series of cross scratches were made, into which the material was rubbed. *Two days* afterwards, the scratches were slightly inflamed. *Three days* afterwards, there was a scab at the seat of inoculation, with slight redness round it. On the *eighth day*, a scab, leaving a very superficial cicatrix, was removed.

Experiment VI.

Vaccinifer — Guinea-pig. Date — October 24, 1885. Vaccine Material — White vaccine.

Exp. VI. Guinea-pig.

Nil.

This guinea-pig was inoculated in the same way with white vaccine cultivation. On the *eighth day*, no local result had been produced. No eruption could be discovered.

Experiment VII.

Vaccinifer — Guinea-pig. Date — October 24, 1885. Vaccine Material — Yellow vaccine.

Exp. VII. Guinea-pig.

Nil.

No local result was produced, and no eruption was observed on the eighth day.

Experiment VIII.

Vaccinifer — Monkey A. Date — November 2, 1885. Vaccine Material — Clear vaccine lymph.

Both arms were shaved, and arm-to-arm vaccination

was performed. Two insertions, by a series of cross scratches, were made on each arm. On the *eighth day*, four typical plump vesicles, with a scab in the centre and raised circumference, resulted. Lymph taken from the vesicles was clear like human lymph. On the *tenth day* the vesicles had scabbed. Cultivations of the lymph showed a white growth.

Experimental vaccination. Exp. VIII. Monkey. Four vaccine vesicles.

Experiment IX.

Vaccinifer—Monkey B. Date—November 16, 1885. Vaccine Material—White variolous cultivation, No. 12.

This was a dark-haired, grey-faced, healthy animal. Both arms were first shaved, and then four insertions of white variola were made, two on each arm, by a series of cross scratches. The material was well rubbed in. No local result was expected to follow; but I predicted an eruption of smallpox of a modified, and probably mild kind. Next day all the marks were scabbed, and the animal was quite well. *Two days* afterwards, it was found asleep, covered up with a blanket. When roused, it looked up quite bright, and scolded me in monkey language. The marks did not look inflamed. On the *fourth day*, a red spot, from which the top had been scratched, was observed on the left cheek. There was another vesicle on the right cheek. There was also a larger vesicle close to the right nipple. The animal was quite well. On the *fifth day*, the vesicles were

Exp. IX. Monkey. White variola. Eight pocks.

Experimental vaccination. Exp. IX. Monkey. White variola. Cultivations.

counted. There were eight in all—one on each cheek, two on the front of the neck, one beside the right nipple, one on the left wrist, and one on each instep. On the *sixth day*, six cultivations from uninjured vesicles were made by Mr Hare, in Koch's nutrient gelatine. The cuticle had been scratched off those on the cheeks and insteps. The temperature was 100°.

Result.

No. II. cultivation showed a white growth, without liquefaction, as seen in Plate XV. Two or three minute orange nodules can be seen also. Nos. 1 and 5 showed a white growth, with ultimate liquefaction. No. 3 showed an orange growth, with liquefaction, and No. 6 a yellow growth with liquefaction. No. 4 gave no reaction. The micrococci were identical with white variola.

Experiment X.

Vaccinifer—Monkey C. Date—November 22, 1885. Vaccine Material — Exposure to infection from monkey suffering from modified variolation.

Exp. X. Monkey. Infection.

To determine whether modified variolation, as described in Experiment IX., is dangerous to the public health, by producing an infectious disease, a third, monkey C, was sent to live with B for a time. After living together for a month, C remained quite well.

EXPERIMENT IX.

WHITE CULTIVATION OF VARIOLA.

Vaccinifer—Monkey. *Vaccine Material*—White variolous cocoon.
Result—Eight pocks. *Time of Cultivation*—Sixth day of eruption.

Both sides of the growth are shewn in the plate.

a White cocoon.
b Deep cadmium or orange growth.
c Diffuse white growth.

a White cocoon.
b Orange cocoon.
c Diffuse white growth.
d Yellow cocoon.

Experiment XI.

Experimental vaccination.

Vaccinifer—Monkey B (Experiment IX.). Date—February 19, 1886. Vaccine Material—Arm-to-arm vaccine lymph.

Exp. XI. Monkey. Vaccination successful after modified variolation.

The object of this experiment was to test whether "white variola" protected from primary vaccination. After an interval of three months, monkey B was vaccinated from arm to arm with vaccine lymph, derived from Case 243, Vaccination Register, 1886, two insertions being made on each arm. On the *fourth day*, the two upper marks showed raised scabs, from beneath which a small quantity of lymph exuded. On the *sixth day*, the scab was easily raised from the vesicle on the left arm, and two test-tubes, containing Koch's nutrient gelatine, were inoculated with the lymph from the raw surface. The upper pock, on the right arm, showed two ill-developed separate vesicles. The lower pocks were papular. On the *ninth day*, there were four dry scabs, corresponding to the four insertions. On the *seventeenth day*, all the scabs were off.

The cultivations liquefied the gelatine.

Experiment XII.

Vaccinifer—Monkey C. Date—February 26, 1886. Vaccine Material—Pure cultivation of white variola, second generation.

Two insertions were made on each arm. On the

Experimental vaccination. Exp. XII. Monkey. Pure white variola. Eruption.

third day, the marks appeared moist. On the *fifth day*, a vesicle appeared on the left cheek, and another on the left arm near the elbow. On the *twelfth day*, there was a distinct scab on the left cheek, while the scab on the elbow was disappearing. On the *eighteenth day*, the scab on the cheek was still very distinct, that on the elbow had disappeared.

Experiment XIII.

Vaccinifer—Monkey D. Date—February 19, 1886. Vaccine Material—Alcoholic wort.

Exp. XIII. Monkey. Fermentation.

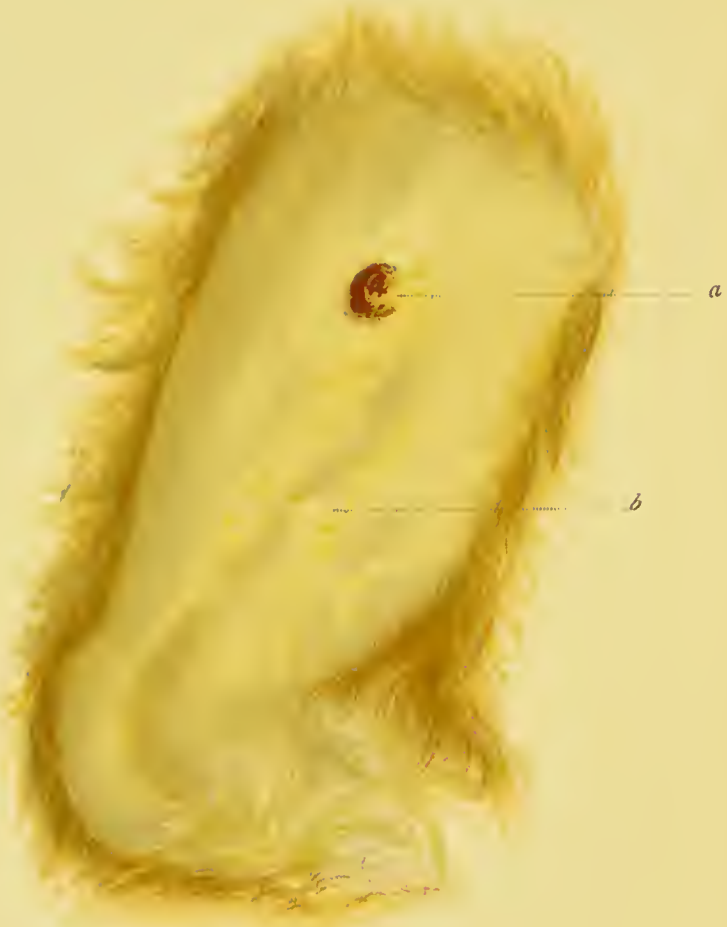
The object of this experiment was to produce fermentation of the blood. Two insertions were made on each arm. No local result was produced. Unfortunately the temperature was not taken.

Experiment XIV.

Vaccinifer—Monkey D. Date—February 26, 1886. Vaccine Material—Pure dried yeast.

Exp. XIV. Monkey. Fermentation.

This monkey was again inoculated at the same points with dried yeast, which was well rubbed in. On the *fourth day*, the animal was feverish, the skin being hot to touch; temperature, 101°·6 Fahr. Raised scabs covered the points of inoculation, and there was apparently some local irritation. On the *fifth day*, temperature 102°, there was a papule on right arm,



EXPERIMENT XIV.

APPARENTLY SUCCESSFUL YEAST INOCULATION.

Time—Eleventh day. *Vaccinifer*—Monkey. *Vaccine Material*—Pure dried yeast. *Result*—Raised circular scab.

Note that three other insertions, one on the same arm, have not taken. The lancet may have been accidentally charged with dried vaccine lymph.

a Upper insertion. *b* Lower insertion.

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corresponding to the upper insertion. On the *sixth day*, temperature 101°, the papule was more distinct. On the *eighth day*, temperature 101°·6, vesicle larger. On the *eleventh day*, vesicle dried on right arm (see Plate XVI.). Experimental vaccination. Exp. XIV. Monkey. Fermentation.

For the sake of brevity I shall describe the animals inoculated with dried yeast as "fermented," and the operation as "fermentation."

Experiment XV.

Vaccinifer — Fermented Monkey D. Date — March 18, 1886. Vaccine Material—Arm-to-arm vaccine lymph.

Four insertions were made as usual. Next day both marks on the left arm were moist-looking; those on the right arm were dry. On the *sixth day*, all the marks were dry; and on the *eighth day*, no result had been produced. The animal was, temporarily at least, insusceptible of the vaccine disease. The result encouraged me to test the validity of this experiment by further observations with dried yeast. Exp. XV. Fermented monkey Unsuccessful vaccination.

Experiment XVI.

Vaccinifer—Modified variolated and vaccinated Monkey B. Date—March 20, 1886. Vaccine Material—Pure dried yeast.

The inoculation was performed as formerly described.

Experimental vaccination. Exp. XVI. Monkey. Fermentation.

On the *fourth day*, temperature 101°·4; doubtful scab on right arm. *Sixth day*—Temperature 101°, the scab has dropped off.

Experiment XVII.

Vaccinifer—Modified variolated Monkey C. Date—March 20, 1886. Vaccine Material—Pure dried yeast.

Exp. XVII. Protected Monkey. Fermentation.

Two insertions were made on each arm. *Fourth day*—One raised papule was seen on each arm. *Sixth day*—There was a raised and prominent scab on each arm. The temperature was 101°. Two cultivations were made from the serum beneath the scabs on the *seventh day*.

Cultivations.

No. 1 showed separate white, yellow, and orange growths. No. 2 showed white and yellow cocoons without liquefaction. The histological appearances of the white and yellow growths are seen in Plate XIV.

Experiment XVIII.

Vaccinifer—Monkey E. Date—April 3, 1886. Vaccine Material—Pure dried yeast.

Exp. XVIII. Monkey. Fermentation.

Two insertions were made on each arm. *Third day*—Right arm, both marks apparently taking; left arm, doubtful. *Fourth day*—Marks distinct on both arms; they look like scabs formed of dried blood, but they differ from these in being somewhat raised, probably

by fluid beneath them. *Fifth day*—Right arm, lower scab most distinct; crust of blood has fallen off the upper insertion, and it appears papular; left arm, both marks show scab on raised papular base. *Eighth day*—Scabs falling off. *Tenth day*—Quite gone. *Twelfth day*—Temperature, 102°·5. *Thirteenth day*—Temperature, 101°·5; blood cultivated. *Sixteenth day*—Quite well. Four tubes of Koch's nutrient gelatine were inoculated from the blood. In one of them a small white cocoon growth appeared, without liquefaction of the gelatine. There was no reaction in the other three. The growth on examination showed micrococci. No cover-glass preparation was made from the blood, which was an unfortunate oversight.

Experimental vaccination. Exp. XVIII. Monkey. Fermentation. Cultivation of blood.

Experiment XIX.

Vaccinifer—Calf C. Date—April 3, 1886.
Vaccine Material—Pure dried yeast.

The object of this and the two following experiments was to ferment the blood of calves. The operation was performed exactly like a vaccination, about fifty insertions being made. *Second day*—Both thighs show the marks of the scratches distinctly. They are not so distinct on the abdomen. *Fourth day*—Marks on right thigh look inflamed. Scattered marks on abdomen show an irritable appearance. The marks on

Exp. XIX. Calf. Fermentation.

Experimental vaccination. Exp. XIX. Calf. Fermentation.

the left thigh, where the scratches were lighter, have died away. *Fifth day*—Five small vesicles, about the size of a millet seed, were observed on the right thigh. Certain of the scratches in this situation continue inflamed. The marks on the left thigh and abdomen are healed. *Eighth day*—Miliary vesicles have disappeared. The temperature was not taken.

Experiment XX.

Vaccinifer—Calf D. Date—April 10, 1886. Vaccine Material—Pure dried yeast.

Exp. XX. Calf. Fermentation.

In order to get a better local effect, the insertions, fifty in number, were made by numerous cross scratches. *Second day*—Marks inflamed. *Third day*—Marks still irritable; temperature, 100°. *Fourth day*—Marks still inflamed. *Fifth day*—Inflammation subsiding, temperature 99°. *Seventh day*—Temperature normal. Three or four raised scabs left. *Ninth day*—All the marks have disappeared. *Eighteenth day*—Small indistinct miliary eruption on thighs and abdomen.

Experiment XXI.

Vaccinifer—Fermented Calf C. Date—April 10, 1886. Vaccine Material—Pure dried yeast.

Exp. XXI. Calf. Refermentation.

Thirty insertions were made by numerous cross scratches; temperature, 100°. On the *third day*, the

marks looked irritable. On the *sixth day*, seven or eight scabs were left, which disappeared by the *eighth day*; temperature, 99°. On the *seventeenth day*, the shaved portion of the abdomen was covered by a miliary eruption. Experimental vaccination. Exp. XXI. Calf. Refermentation.

Both calves were then sent to London to be vaccinated from calf-to-calf by Dr Cory, to whom I feel much indebted for his kindness.

Experiment XXIV.

Vaccinifer—Fermented Monkey E. April 22, 1886. Vaccine Material — Arm-to-arm vaccine lymph.

Four insertions were made on the left arm. *Fifth day*—One insertion appears to be taking; temperature, 102°. *Seventh day*—Beautiful typical Jennerian pock, circular, plump, and umbilicated. It had developed completely in forty-eight hours. *Thirteenth day*—Dry black scab. The other three insertions did not take. Exp. XXIV. Fermented monkey. Vaccination.

Experiment XXXIII.

Vaccinifer—Twice fermented Calf C. Date—May 4, 1886. Vaccine Material—Calf-to-calf vaccine lymph.

This calf was vaccinated by Dr Cory at the National Vaccine Establishment. Its weight was 124 lbs., and its temperature, 102°, on the day of operation. Thirty- Exp. XXXIII. Fermented Calf. Vaccination.

Experimental vaccination.

Exp. XXXIII. Fermented calf. Vaccination.

six insertions were made in the usual way, and it was then returned to me. *Fourth day*—All the marks vesicular. *Fifth day*—Calf lymph taken. *Sixth day*—Vesicles much larger and drying. *Seventh day*—Vesicles scabbed. Cast taken in plaster of Paris by Dr Caird.

Experiment XXXIV.

Vaccinifer—Fermented Calf D. Date—May 4, 1886. Vaccine Material—Calf-to-calf vaccine lymph.

Exp. XXXIV. Fermented calf. Vaccination.

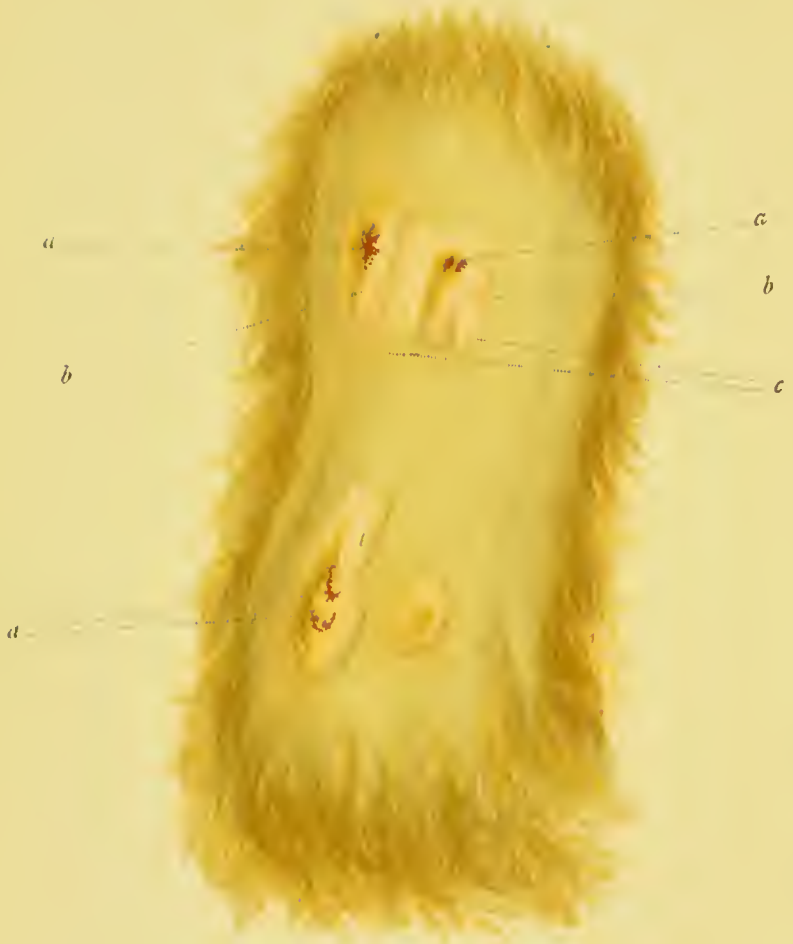
This animal was also vaccinated by Dr Cory at the National Vaccine Establishment. Its weight was 105 lbs., and its temperature 102°, on the day of operation. Thirty-seven insertions were made in the usual manner.

Fourth day—All the marks successful. *Fifth day*—Lymph taken. *Seventh day*—Vesicles scabbing. Cast in plaster of Paris taken by Dr Caird. At the end of a fortnight both calves were quite well, but the scabs had not come off.

Experiment LI.

Vaccinifer.—Monkey R. Date—June 12, 1886. Vaccine Material—Clear variolous lymph from Case H.

Confluent smallpox (unvaccinated); sent by Mr Bott



EXPERIMENT LI.

VARIOLOUS INOCULATION.

Time—Eighth day. *Vaccinifer*—Unprotected Monkey. *Vaccine Material*—Clear variolous lymph from Case H, Hospital Ships. *Result*—Four large vesicles, with areola. No secondary eruption.

a Central umbilication.
b Pale raised zone.
c Areola.

EXPERIMENT LII.

VARIOLOUS INOCULATION.

Time—Eighth day. *Vaccinifer*—Unprotected Monkey. *Vaccine Material*—Opaque variolous lymph, Case E, Hospital Ships. *Result*—Four Vesicles. No secondary eruption.

a Central dark umbilication.
b Raised zone of paler colour.
c Areola.

from the Hospital Ships, Purfleet. Four insertions were made on the right arm; temperature 98°·5. On the *eighth day* the temperature was 101°, and four good-sized vesicles had developed. There was no secondary eruption. On the *eleventh day*, four secondary papules appeared on the right hip and sacrum; temperature 101°. On the *fourteenth day*, temperature 99°; both vesicles and papules had scabbed. *Twenty-first day*—Scabs healed; marks of cicatrices left; temperature normal. Experimental vaccination. Exp. LI. Monkey. Variolation.

Experiment LII.

Vaccinifer—Monkey S. Date—June 12, 1886. Vaccine Material—Stored opaque variolous lymph from Case C.

Temperature normal, 98°·5. Four insertions were made on the right arm. *Eighth day*—Four good vesicles; temperature 101°·4. *Eleventh day*—No secondary eruption could be discovered. *Fourteenth day*—Vesicles scabbed. *Twenty-first day*—Healed. Exp. LII. Monkey. Variolation.

Experiment XXII.

Vaccinifer — Unprotected Monkey F. Date—April 10, 1886. Vaccine Material—Pure dried yeast.

Two insertions were made on each arm; temperature

Experimental vaccination. Exp. XXII. Monkey. Fermentation.

normal, 98°·5. *Fourth day*—Temperature 101°; all the marks taking, and scabs raised above the surface of the surrounding skin. *Sixth day*—Temperature 101°. *Seventh day*—Cultivation of blood. *Ninth day*—Scabs on right arm becoming indistinct: scabs on left arm still raised. *Sixteenth day*—Two scabs removed from left arm, leaving indistinct cicatrices.

Result of Cultivations—Eleventh day.

1. Tube of Koch's nutrient gelatine showed separate white, yellow, and orange growths.
2. Tube of Koch's nutrient gelatine showed white and yellow cocoons.

Cultivations.

Microscopic examination of the white growth showed numerous spherical micrococci, arranged in chains, pairs, packets, and groups. The organisms were apparently undergoing rapid fission.

The yellow growth presented appearances very similar to the white, but the organisms were somewhat larger.

Unfortunately, cover-glass preparations were not made from the fresh blood.

Experiment XXIII.

Vaccinifer — Unprotected Monkey G. Date — April 10, 1886. Vaccine Material—Pure dried yeast.

Temperature 98°·5. Four insertions were made, two

on each arm. *Fourth day*—All marks distinct, and two of them were raised above the surrounding surface. *Fifth day*—Temperature 101°·4. *Sixth day*—Cultivation of blood. *Ninth day*—One distinct scab on right, and another on the left arm. *Sixteenth day*—Scab on left arm still adherent, and was removed next day. Experimental vaccination. Exp. XXIII. Monkey. Fermentation.

Result of Cultivations:—

No. 1 showed two minute white cocoons in Koch's nutrient gelatine.

No. 2 gave no result.

Experiment XXIV.

H. monkey died the day after inoculation with pure dried yeast. It was weakly.

Experiment XXV.

Vaccinifer—Unprotected Monkey I. Date—April 27, 1886. Vaccine Material—Pure dried beer yeast.

Two insertions were made on right arm. *Second day*—Very slightly raised scabs. *Fourth day*—Temperature 99°; scabs falling off. *Sixth day*—Quite well; no effect apparent. Exp. XXV. Monkey. Fermentation.

Experimental vaccination.

Experiment XXVI.

Vaccinifer—Unprotected Monkey K. Date—April 27, 1886. Vaccine Material—Pure dried beer yeast.

Exp. XXVI. Monkey. Fermentation.

Two insertions were made on the left arm. *Sixth day*—Slightly raised scabs; temperature 100°. *Eighth day*—All scabs off; animal well.

Experiment XXVII.

Vaccinifer—Unprotected Monkey L. Date—April 27, 1886. Vaccine Material—Pure dried beer yeast.

Exp. XXVII. Monkey. Fermentation.

Two insertions were made on the right arm. *Second day*—Indistinct scabs. *Fourth day*—Temperature 102°; all scabs off. *Sixth day*—Temperature 102°. *Eighth day*—No further notes of temperature; animal apparently well.

Experiment XXVIII.

Vaccinifer—Unprotected Monkey M. Date—April 27, 1886. Material—Pure dried beer yeast.

Exp. XXVIII. Monkey. Fermentation.

Fourth day—Indistinct scabs, temperature 102°. *Sixth day*—Arm well; temperature 101°. *Eighth day*—Animal apparently well.

Experiment XXIX. Experimental vaccination.

Vaccinifer—Unprotected Monkey N. Date—April 27, 1886. Vaccine Material—Pure dried beer yeast.

Fourth day—Two large raised scabs; temperature 101°. *Sixth day*—Picked off the scabs, and found cicatrices of lancet healed; temperature 101°. *Eighth day*—Quite well. Exp. XXIX. Monkey. Fermentation.

Experiment XLVII.

Vaccinifer—Unprotected Monkey Q.[1] *Date—May 29, 1886. Vaccine Material—Pure dried yeast.*

Sixth day—Local raised scabs resulted. *Ninth day*—Found dead. Exp. XLVII. Monkey. Fermentation.

Experiment XXX.

Vaccinifer—Fermented Monkey F (Exp. 22). Date—April 27, 1886. Vaccine Material—Arm-to-arm variolous lymph from confluent case.

Four insertions were made, two on each arm.

[1] Seven other monkeys belonging to the same lot as Q died before being inoculated. It is probable that they succumbed to the sudden change from a warm to a cold climate.

Experimental vaccination. Exp. XXX. Monkey. Variolation.

Fourth day—Temperature 99°; four vesicles. *Fifth day*—Temperature 101°; vesicles somewhat larger. *Sixth day*—Vesicles flattened, and not so prominent. *Seventh day*—Temperature 101°·5; all pocks show a red areola. *Eighth day*—Pocks well raised and distinct, containing purulent material. *Ninth day*—Scabbing; temperature 101°. *Eleventh day*—All scabs off, leaving ulcerated surfaces. *Thirteenth day*—Secondary scabs have formed; no secondary eruption.

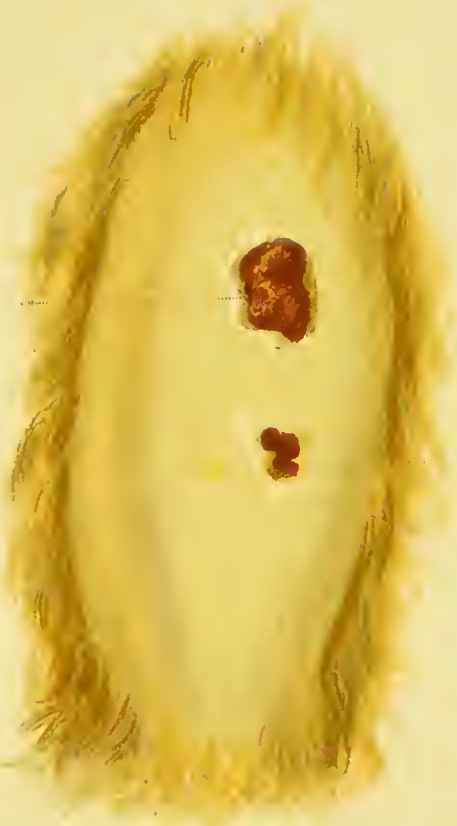
Experiment XXXI.

Vaccinifer—Fermented Monkey G (Exp. 23). Date—April 27, 1886. Vaccine Material—Arm-to-arm variolous lymph (Confluent).

Exp. XXXI. Monkey. Variolation.

Four insertions were made, two on each arm. *Second day*—Temperature 100°. *Fifth day*—Distinct vesicles of considerable size on each arm; there was a scab in the centre of each, surrounded by a raised white zone; temperature 101°·5. *Fifth day*—Zone not so distinct; vesicles flatter and larger. *Seventh day*—All the pocks show a red areola; temperature 101°·5. *Ninth day*—Scabs. *Eleventh day*—All the scabs removed except one, probably by scratching. *Thirteenth day*—No secondary eruption was discovered.[1]

[1] Monkey O died before being inoculated.



EXPERIMENT XXXI.

VARIOLOUS INOCULATION.

Time—Sixteenth day. *Vaccinifer*—Fermented Monkey. *Vaccine Material*—Arm-to-arm variolous lymph. *Result*—Four large vesicles, with areola. No secondary eruption.

a Primary scab.
b Secondary scab.
c Cicatrix.

EXPERIMENT XXXVI.

VARIOLOUS INOCULATION.

Time—Eighth day. *Vaccinifer*—Fermented Monkey. *Vaccine Material*—Arm-to-arm variolous lymph. *Result*—Four vesicles, with slight areola. No secondary eruption.

a Central part of vesicle.
b Pale raised zone.
c Areola.

Experiment XXXV.

Experimental vaccination.

Vaccinifer—Fermented Monkey M (Exp. 28). Date—May 5, 1886. Vaccine Material—Pure dried yeast.

I considered the first inoculation unsatisfactory, and repeated it, to make sure of the maximum protection from fermentation. Two insertions were made on the right arm. *Third day*—Marks slightly irritable; temperature 100°·2. *Fifth day*—Scabs disappearing. *Eighth day*—Quite well.

Exp. XXXV. Monkey. Fermentation.

Experiment XXXVI.

Vaccinifer—Fermented Monkey N. Date—May 5, 1886. Vaccine Material—Arm-to-arm variolous lymph.

Four insertions were made on the right arm; temperature 101°·5. *Fourth day*—Four black scabs. *Eighth day*—Raised white zone round scabs, but vesicles are causing little irritation; they look exactly like vaccine vesicles, their size and plumpness are deficient, considering the quantity of material used. *Tenth day*—All marks successful. *Twelfth day*—Good vesicles. *Fourteenth day*—All scabbed; no secondary eruption. *Twentieth day*—All scabs off; quite well.

Exp. XXXVI. Monkey. Variolation.

Experimental vaccination.

Experiment XXXVII.

Vaccinifer—Fermented Monkey L. Date—May 5, 1886. Vaccine Material—Arm-to-arm variolous lymph.

Exp. XXXVII. Monkey. Variolation.

Four insertions were made on right arm; temperature 100°. *Fourth day*—Temperature 101°; all marks quiet, and apparently not taking. *Fifth day*—Temperature 102°; still no appearance of taking. *Eighth day*—Only one vesicle; much modified. *Tenth day*—All marks distinctly vesicular. *Twelfth day*—Four pocks exactly like modified vaccine vesicles; no secondary eruption. *Fourteenth day*—Vesicles dry and scabbed; scabs off; quite well.

Experiment XXXVIII.

Vaccinifer—Fermented Monkey K. Date—May 5, 1886. Vaccine Material—Arm-to-arm variolous lymph.

Exp. XXXVIII. Monkey. Variolation.

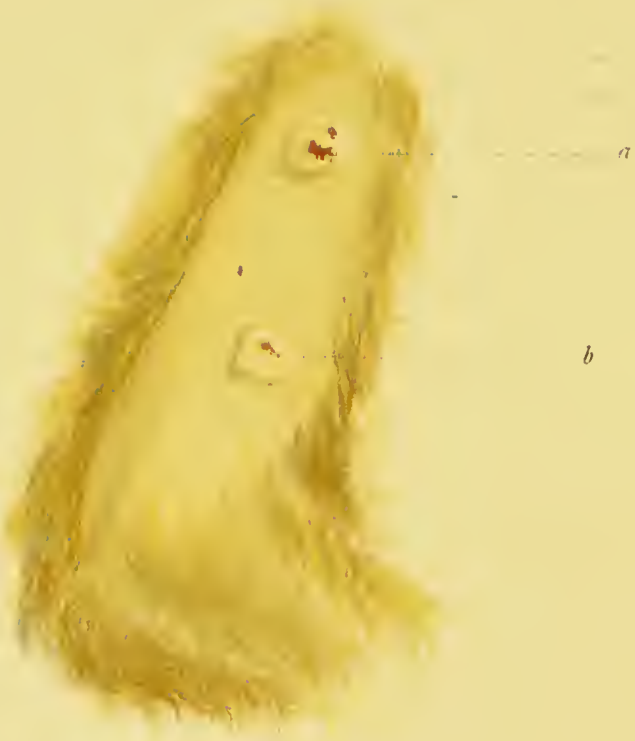
Four insertions were made on the left arm. Temperature, 99°. *Fourth day*—All marks taking. *Eighth day*—Four modified vesicles. *Tenth day*—Four fairly good vesicles, without areola. *Twelfth day*—No secondary eruption.

EXPERIMENT XXXVIII.

VARIOLOUS INOCULATION.

Time—Eighth day. *Vaccinifer*—Fermented Monkey. *Vaccine Material*—Arm-to-arm variolous lymph. *Result*—Four vesicles, with slight areola. No secondary eruption.

a Umbilication.
b Pale raised zone of vesicle.
c Areola.

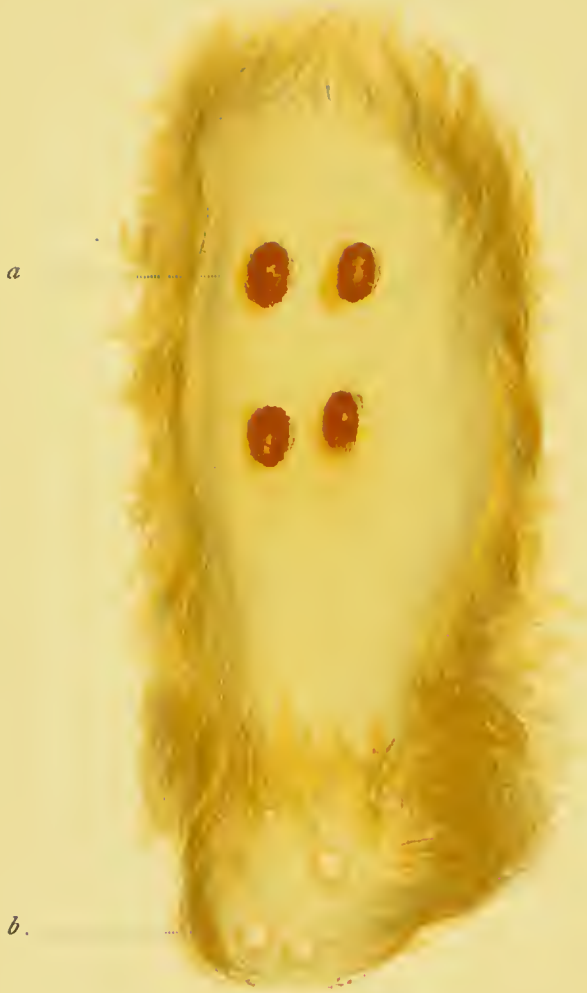


EXPERIMENT XXXIX.

VARIOLOUS INOCULATION.

Time—Eighth day. *Vaccinifer*—Fermented Monkey. *Vaccine Material*—Arm-to-arm variolous lymph. *Result*—Two small vesicles, without areola. No secondary eruption.

a Central umbilication.
b Pale raised zone of vesicle.



EXPERIMENT XLVI.

VARIOLOUS INOCULATION.

Time—Thirteenth day. *Vaccinifer*—Unprotected Monkey. *Vaccine Material*—Clear variolous lymph from Case I, Hospital Ships. *Result*—Four dark oval scabs at the points of inoculation. Three secondary vesicles, two days old, on elbow.

a Primary local scabs.
b Secondary vesicles.

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Experiment XXXIX. Experimental vaccination.

Vaccinifer—Fermented Monkey I. Date—May 5, 1886. Vaccine Material—Arm-to-arm variolous lymph.

Two insertions were made on the right arm. *Eighth day*—Two modified vesicles. *Tenth day*—Good vesicles. *Twelfth day*—No secondary eruption. *Fourteenth day*—Scabs quite dry. *Twenty-first day*—All scabs off. Exp. XXXIX. Monkey. Variolation.

Experiment XLVI.

Vaccinifer—Unprotected Monkey P. Date—May 29, 1886. Vaccine Material—Clear variolous lymph from Case I., Purfleet.

Four insertions were made on right arm. Temperature, 99°·5. *Fourth day*—Temperature 100°·5. Vesicles forming. *Eighth day*—Distinctly formed vesicles. *Tenth day*—Temperature 101°. Vesicles increased, with pink areola surrounding them. *Twelfth day*—Four small secondary vesicles on right elbow. *Thirteenth day*—Areola has disappeared, and vesicles are scabbing. Secondary vesicles are drying up. *Fifteenth day*—Temperature 100°; marks dry; secondary scabs have nearly disappeared. Exp. XLVI. Monkey. Variolation.

Experimental vaccination.

Experiment XLIII.

Vaccinifer—Fermented and Vaccinated Monkey E. Date—May 23, 1886. Vaccine Material —Arm-to-arm variolous lymph.

Exp. XLIII. Monkey. Variolation.

This monkey had been successfully vaccinated a month previously. Four insertions were made on the right arm. No result was produced.

Experiment XLIV.

Vaccinifer—Twice fermented and vaccinated Monkey M. Date—May 23, 1886. Vaccine Material—Arm-to-arm variolous lymph.

Exp. XLIV. Monkey. Variolation.

This monkey had been vaccinated ten days previously. *Fourth day*—Variolous marks papular. Vaccine marks scabbed. *Eighth day*—No further result.

VACCINATION AFTER FERMENTATION AND VARIOLATION.

Experiments XLI. (*May 19, 1886*), **XLII.** (*May 20, 1886*), **XLVIII., XLIX.,** and **L.** (*May 30, 1886*).

Vaccination after fermentation and variolation.

Vaccinifers—Monkeys F, G, N, I, and L, were vaccinated with stored vaccine lymph, after fermentation and variolation, without result.

EXPERIMENT XLV.

EFFECT OF YEAST INOCULATION ON ADULT HUMAN SKIN.

Time—Eighth day. *Vaccinifer*—Protected by vaccination and re-vaccination. *Vaccine Material*—Pure dried yeast. *Result*—Raised scabs, with areola.

Note that the scabs were more raised, and the areola more distinct on the sixth and seventh days.

a Raised scab.
b Areola.

a Raised scab.
b Areola.

Vaccination after Simple Variolation.

Experimental vaccination.

Experiment LIII.

Vaccinifer—Variolated Monkey R. Date—July 10, 1886. Vaccine Material—Stored vaccine lymph.

Vaccination after simple variolation.

One insertion was made on the right arm. *Fourth day*—Vaccination taking. *Eighth day*—Good vesicle, size of threepenny piece. *Eleventh day*—Distinct typical scab, the same as in Plate II.

Fermentation after Vaccination.

Experiment XLV.

Date—May 28, 1886.

Fermentation after vaccination.

I inoculated myself with pure dried yeast, by two insertions on the left arm. *Second day*—Both marks distinctly inflamed. *Sixth day*—Raised scab, with inflammation surrounding it. Scab attained its greatest thickness. *Eighth day*—Scab and redness still persistent (see Plate XXIV.). *Ninth day*—Inflammation subsiding. *Tenth day*—Scab becoming smaller. *Sixteenth day*—Distinct cicatrices after removal of scabs. There was no rise of temperature during the experiment.

Experimental vaccination.

Experiment LIV.

Vaccinifer—Vaccinated and Variolated Monkey R. Date—August 22, 1886. Vaccine Material—Pure dried yeast. Temperature —98°·5.

Second day—Temperature 101°·5. *Third day*—Scratches look moist; temperature 100°·4. *Fourth day*—Temperature 100°. *Sixth day*—Temperature 100°; marks dry. *Eighth day*—Temperature 99°.

TABLE *showing the Results of Experimental Vaccination.*

Results of experimental vaccination.

Vaccinifers.

No.	Date.	Vaccinifer.	Material.	Result.
1	27/8/85.	Calf A.	White, yellow, and orange vaccine.	Eruption on head.
2	8/9/85.	Calf B.	Opaque variolous lymph.	Eruption on back.
3	29/9/85.	Calf A.	Variolous lymph.	Protected by vaccine, I.
4	29/9/85.	Calf B.	Opaque variolous lymph.	Protected by variolation, II.
5	24/10/85.	Guinea-pig.	Orange vaccine.	Local scab.
6	24/10/85.	Do.	White vaccine.	No local result. Constitutional ?
7	24/10/85.	Do.	Yellow vaccine.	No local result. Constitutional ?
8	2/11/85.	Monkey A.	Arm-to-arm vaccine lymph.	Four typical vaccine vesicles.
9	16/11/85.	Monkey B.	White variola.	Eight pocks. No local result.
10	22/11/85.	Monkey C.	Infection of modified variola.	No effect produced.
11	19/2/86.	Monkey B.	Arm-to-arm vaccine lymph.	Modified vaccination.
12	26/2/86.	Monkey C.	White variola—2d generation.	Two small vesicles. No local result.
13	19/2/86.	Monkey D.	Alcoholic wort.	No result.
14	26/2/86.	Do.	Pure dried yeast.	Fever and local scab.
15	18/3/86.	Do.	Arm-to-arm vaccine lymph.	Protected. No effect.
16	20/3/86.	Monkey B.	Pure dried yeast.	Fever and local scabs.
17	20/3/86.	Monkey C.	Do.	Do.
18	3/4/86.	Monkey E.	Do.	Do.
19	3/4/86.	Calf C.	Do.	Local miliary eruption.
20	10/4/86.	Calf D.	Do.	Do.
21	10/4/86.	Calf C.	Do.	Do.
24	22/4/86.	Monkey E.	Arm-to-arm vaccine lymph.	One typical vesicle.
33	4/5/86.	Calf C.	Calf-to-calf vaccine lymph.	Successful vaccination.
34	4/5/86.	Calf D.	Do.	Successful vaccination.
51	12/6/86.	Monkey R.	Clear stored variolous lymph.	Four local pocks. No secondary eruption.

Results of experimental vaccination.

Vaccinifers.

Results of Vaccination—continued.

No.	Date.	Vaccinifer.	Material.	Result.
52	12/6/86.	Monkey S.	Opaque stored variolous lymph.	Four local pocks.
22	10/4/86.	Monkey F.	Pure dried yeast.	Fever and raised local scabs.
23	10/4/86.	Monkey G.	Do.	Do.
25	27/4/86.	Monkey I.	Pure dried beer yeast.	Slight scabbing.
26	27/4/86.	Monkey K.	Do.	Do.
27	27/4/86.	Monkey L.	Do.	Fever and slight scabs.
28	27/4/86.	Monkey M.	Do.	Fever.
29	27/4/86.	Monkey N.	Do.	Fever and raised scabs.
47	29/5/86.	Monkey Q.	Pure dried yeast.	Local raised scabs.
30	27/4/86.	Monkey F.	Arm-to-arm variolous lymph.	Four local pocks. No secondary eruption.
31	27/4/86.	Monkey G.	Do.	Four local pustules.
35	5/5/86.	Monkey M.	Pure dried yeast.	Slight fever.
35a	13/5/86.	Do.	Stored vaccine lymph.	Successful vaccination.
36	5/5/86.	Monkey N.	Arm-to-arm variolous lymph.	Four local vesicles. No secondary eruption.
37	5/5/86.	Monkey L.	Do.	Four modified pocks. No secondary eruption.
38	5/5/86.	Monkey K.	Do.	Four modified vesicles. No secondary eruption.
39	5/5/86.	Monkey I.	Do.	Two local vesicles. Small secondary vesicles.
46	29/5/86.	Monkey P.	Clear stored variolous lymph.	Four local vesicles.
43	23/5/86.	Monkey E.	Arm-to-arm variolous lymph.	No result. Protected.
44	23/5/86.	Monkey M.	Do.	No result.
41	19/5 86.	Monkey F.	Stored vac. lymph.	Protected.
42	20/5/86.	Monkey G.	Do.	Do.
48	30/5/86.	Monkey N.	Do.	Do.
49	30/5/86.	Monkey I.	Do.	Do.
50	30/5/86.	Monkey L.	Do.	Do.
53	10/7/86.	Monkey R.	Do.	Successful vaccination.
45	28/5/86.	Man.	Pure dried yeast.	Raised scabs. No fever.
54	22/8/86.	Monkey R.	Do.	Fever.

TABLE *showing the Results of Experimental Inoculation with different Vaccine Materials.*

Results of experimental vaccination.

Vacciue materials.

Vacciue Material.	Vaccinifer.	Resnlt.
Arm - to - arm vaccine lymph.	Unprotected monkey A.	Successfnl vaccination.
Do.	Modified variolated monkey B	Modified vaccination.
Do.	Fermented monkey D.	Protection.
Do.	Do. monkey E.	Modified vaccination.
Calf - to - calf vaccine lymph.	Do. calf C.	Successful vaccinatiou.
Do.	Do. calf D.	Do.
Stored vaccine lymph.	Fermented and variolated monkey F.	Protected.
Do.	Fermented and variolated monkey G.	Do.
Do.	Fermented and variolated monkey N.	Do.
Do.	Fermeuted and variolated monkey I.	Do.
Do.	Fermented and variolated monkey L.	Do.
Do.	Variolated monkey.	Successful vaccination.
Arm-to-arm variolous lymph.	Fermented monkey F.	Local pocks.
Do.	Do. monkey G.	Do.
Do.	Do. monkey N.	Local vesicles.
Do.	Do. monkey L.	Do.
Do.	Do. monkey K.	Do.
Do.	Do. monkey I.	Do.
Do.	Fermentcd and vaccinated monkey E.	Protected.
Do.	Fermented and vaccinated monkey M.	Protected. No local result.
Stored variolous lymph.	Unprotected calf B.	Eruptiou on back.
Do.	Variolated calf B.	Protected.
Do.	Modified vaccinatcd calf A.	Protcctcd. No local result.
Do.	Unprotcctcd monkcy R.	Local pocks.
Do.	Do. monkey S.	Do.
Do.	Do. moukey P.	Local and sccondary pocks.
Vaccine cultivations.	Unprotcctcd calf A.	Eruptiou on head.
Orange do.	Guinea-pig.	Local scab. Constitutional?

Results of experimental vaccination.

Vacciue materials.

Results of Vaccine Materials—continued.

Vaccine Material.	Vaccinifer.	Result.
White cultivation.	Guinea-pig.	No local result. Constitutional ?
Yellow do.	Do.	No local result.
White variolous cultivation.	Unprotected moukey B.	No local result. General cruption.
White variolous infectiou.	Do. monkey C.	No infection.
Pure white variolous cultivation.	Monkey C.	No local result. General eruption.
Alcoholic wort.	Unprotected monkey D.	No result.
Pure dried yeast.	Monkey D.	Fever aud local vesicle. Accidental vaccination.
Do.	Modified variolated monkey B.	Fever and local scabs.
Do.	Modified variolated monkey C.	Do.
Do.	Unprotected monkey E.	Do.
Do.	Do. calf C.	Local miliary eruption.
Do.	Do. calf D.	Do.
Do.	Fermented calf C.	Do.
Do.	Unprotected moukey F.	Fever and raiscd local scabs.
Do.	Do. monkey G.	Do.
Pure dried beer yeast.	Do. monkey I.	Slight scabbing.
Do.	Do. monkey K.	Do.
Do.	Do. monkey L.	Fever and slight scabs.
Do.	Do. monkey M.	Fever.
Do.	Do. monkey N.	Fever aud raised scabs.
Do.	Do. monkey Q.	Local raiscd scabs.
Pure dricd yeast.	Fermented monkey M.	Slight fever.
Do.	Variolated aud vaccinatcd moukcy R.	Do.
Do.	Vaccinated man.	Raiscd scabs. No fever.

GENERAL SUMMARY.

Results of experimental vaccination.

Vaccine Materials.

Vaccine materials.

1. Arm-to-Arm Vaccine Lymph.
2. Calf-to-Calf Vaccine Lymph.
3. Stored Vaccine Lymph.
4. Arm-to-Arm Variolous Lymph.
5. Stored Variolous Lymph.
6. Vaccine Cultivations.
7. Variolous Cultivations.
8. Liquid Yeast.
9. Pure Dried Yeast.

Vaccinifers.

Vaccinifers.

1. Unprotected Calves.
2. Unprotected Guinea-Pigs.
3. Unprotected Monkeys.
4. Fermented Calves.
5. Fermented Monkeys.
6. Vaccinated Monkeys.
7. Variolated Monkeys.
8. Modified Variolated Monkeys.
9. Fermented and Vaccinated Monkeys.
10. Fermented and Variolated Monkeys.
11. Vaccinated and Variolated Monkeys.

Results of experimental vaccination.

GENERAL SUMMARY—*continued.*

Results.

I. Constitutional.

I. *Constitutional.*

1. Fever without Local Lesion.
2. Fever with Local Lesion.
3. Fever with Eruption.
4. Protection.

II. Local.

II. *Local.*

1. Simple Raised Scabs.
2. True Vesicles or Pustules.
3. Modified Vesicles or Pustules.
4. Miliary Eruption.
5. No Local Lesion.

CHAPTER IX.

RELATION OF FERMENTATION TO VACCINIA AND VARIOLA.

Acid fermentation of lymph.

THE fact that alkaline vaccine lymph undergoes acid fermentation when stored in tubes, and the conviction that the peculiar spherical bodies, like oil drops, described by Cohn and Burdon Sanderson, were really cells of a species of yeast, led me to investigate the possible pathogenic properties of the torula cerevisiæ. Pfeifer (*loc. cit.*) has observed a "Saccharomyces Vaccinæ" in calf lymph, which he considers to be an accidental contamination of the lymph. But, even assuming this to be the case, it is a remarkable fact that such yeast or torula cells can exist in such material. If they really can do so, it is possible that they may be able to develop in the living animal body. I had come to the conclusion that these bodies, from their frequent occurrence, could not always be accidental, and that, as opacity of lymph was due to the development in it of the spores in clear lymph, these peculiar bodies might be resting forms, probably containing spores. As has been stated already, they are best examined in the

Saccharomyces vaccinæ.

Saccharomyces vaccinæ.

fresh condition, so that any fallacy arising from the mode of demonstration is thereby avoided. They also stain very easily, and possess a very delicate envelope or cell wall. This evanescent characteristic distinguishes them from the red and white blood-corpuscles, and when we take into account their gradual development, as observed by Cohn, and their numbers and smaller size, there can be no doubt that they are different from blood-corpuscles. I have never seen blood cells in such numbers in any specimen of lymph I have examined. The hypothesis that these forms simply follow, and do not develop from micrococci, involves the admission of the contamination theory advanced by Pfeifer. I think that this is negatived by their constant occurrence, as described by independent observers.

Artificial cultivation of yeast.

That some micrococci may be embryonic forms of yeast is rendered probable by the appearances observed when we cultivate yeast artificially. Pure yeast does not liquefy Koch's nutrient gelatine, and the growth is white. When examined microscopically, the yeast cells are much smaller than those used for starting the growth, and resemble large micrococci. If the gelatine liquefy, however, the cells become larger, and this increase of size also takes place in pure cultivations in more suitable soil. Then, when a well-fed organism is suddenly starved (which is the moment of reproduction), variations are observed to take place in other cases. Analogous developmental conditions occur also in the course of the life history of bacteria. I may mention

also, that change of colour frequently takes place in ripening fungi; and that change of habit with environment may also take place in other fungi; reproduction being rare in many cases. Although many botanists separate bacteria from yeasts, Sachs classes them together as Protophyta. Bacteria and yeasts.

The point to which my inquiries were directed was whether some of the globular bacteria or micrococci in vaccine lymph were not really embryonic forms of yeast, and whether yeast possessed any pathogenic properties.

Turning from works on botany to those on pathology I found that nothing positive was known on the subject. Wagner (*General Pathology*) states that its pathological significance is still doubtful, and Ziegler (*General Pathological Anatomy*) states that yeast possesses little pathological significance. Professor Tyndall (*Floating Matter of the Air*), however, does not seem to share this last opinion, for he quotes the following passage, from an essay on the Pathological Part of Physic, by Robert Boyle:—"And let me add that he that thoroughly understands the nature of ferments and fermentations, shall probably be much better able, than he that ignores them, to give a fair account of divers phenomena of several diseases (as well fevers as others), which will perhaps be never properly understood without an insight into the doctrine of fermentations." Pathogenic properties of yeast. Wagner. Ziegler. Boyle

Pasteur (*Studies on Fermentation*, p. 144) states that vegetation of yeast, in a medium adapted to its Pasteur.

Properties of yeast. teur.

nutrition, can occur at any temperature between zero and 55° C. (131° F.), although a temperature between 15° C. and 30° C. (59° and 86° F.) is the most favourable to its occurrence. He holds that "the fermentative character is not an invariable phenomenon of yeast life. Yeast is a plant which does not differ from ordinary plants. It manifests its fermentative power solely in consequence of particular conditions under which it is compelled to live. It may carry on its life as a ferment or not, and, after having lived without manifesting the slightest symptom of fermentative character, it is quite ready to manifest that character when brought into suitable conditions.

"The fermentative character, therefore, is not a power peculiar to cells of a special nature. It is not a permanent character of a particular structure, like, for instance, the property of acidity or alkalinity. It is a peculiarity dependent on external circumstances, and on the nutritive conditions of the organism" (p. 266).

Resting stage of bacteria. Koch.

With regard to the resting stage of bacteria, Koch (*Traumatic Infective Diseases*, p. 47) says:—"It is very probable that these micrococci (*i.e.*, in spreading abscess of rabbits), like other bacteria, form resting spores after the expiration of their vegetative life," and also that "the assumption (made by himself) that the micrococci in the cheesy contents of abscesses are dead, does not appear in keeping with the result of inoculation."

Ewart.

Ewart (*Proc. Roy. Soc. Lond.*, xxvii.) concludes "that Billroth was probably right in believing that micro-

cocci were the spores of ordinary bacteria." Ziegler states that it is not yet certain whether or not the micrococci produce spores. Klein is certain that "micrococci propagate always by simple division, never by any other means, *e.g.*, gemmation and spores. All assertions to the contrary are based on incorrect observations." I conclude, therefore, that a difference of opinion exists among bacteriologists as to the mode of reproduction of micrococci.

Resting stage of bacteria. Ziegler. Klein.

Koch is probably right when he says that a particular form of bacterium corresponds to each infective disease, and that opinion is quite consistent with the view that such a bacterium may present growth-forms or developmental conditions in the course of its life history. Under the same conditions, *i.e.*, at the same stage of the disease, its form is always the same. The form of bacterium present in clear vaccine lymph is a very minute spore, ·15 μ in diameter, and if the lymph be dried immediately the further development of the spore is prevented. Its activity can be revived by moistening the dried film of lymph with water, and this material, when properly employed, produces perfect vaccination. Between this minute body and the large torula (2 μ to 5 μ) cell in opaque lymph, there is apparently no more connection than there is between an acorn and an oak, but there are certain close analogies between vaccine lymph and yeast.

Bacteria forms. Koch.

I am indebted to Mr Duncan M'Glashan, Caledonian Distillery, for much practical information about yeast.

Practical information about yeast. M'Glashan.

The manufacture of yeast is an important branch of trade, both on the Continent and in this country. In the moist condition it soon "goes bad," but, if carefully collected and dried by compression, it may be kept for weeks, if protected from the atmosphere, without losing its power of exciting fermentation. Mr M'Glashan informs me that the smugglers in the Highlands in former times were aware that yeast, if protected from damp, could be preserved in a dry state. When about to stop their illicit distillation for the season, they collected a quantity of dry heather, which was tied into bunches. The fermenting wort was then stirred with these, till as much yeast as possible adhered to the small branches of the heather, and the bunches were then dried and hung up in their cottages during the winter. Next season fermentation was started again by simply throwing these into the wort. Pasteur (*op. cit.*) states that yeast is able to grow fairly well at the temperature of the body, and it was therefore a suitable vaccine material, it only being necessary to obtain it in the dried state. The appearance of the material used for inoculation is seen in Plate XIII. Yeast, therefore, like vaccine lymph, can be stored dry; it can be revived by suitable moisture and temperature; it soon undergoes putrefaction if stored moist; it can grow in the body when inoculated, producing a febrile condition in monkeys; and cultivations of micrococci have been obtained from the blood of monkeys after inoculation with dried

Pasteur.

Its properties.

yeast. This last result is impossible a fortnight after the inoculation. I have been prevented from carrying out experiments on the acclimatisation of yeast in the animal body by a dearth of monkeys. It would be interesting to know whether the lymph exuding from the fresh surface beneath a growing yeast scab, would produce a vesicle if inoculated in another animal. Inoculation of yeast.

Yeast also agrees with vaccine and variolous cultivations in producing a constitutional result, the temporary febrile condition being probably due to the growth of the ferment in the blood. The febrile condition invariably occurred after inoculation. Does fermentation of the blood of monkeys, by means of inoculation with dried yeast, protect them from vaccination and variolation? My experiments show that it does not do so entirely, but it certainly has a remarkable effect, in monkeys, in modifying the action of both the vaccine and variolous virus. My first experiment appeared to show that inoculation with dried yeast is perfectly protective from arm-to-arm vaccination. But repeated control experiments led me to the conclusion that the first monkey (Plate XVI.) was, perhaps, vaccinated accidentally with a lancet charged with dried lymph. The other experiments were performed with a new lancet, and this was never afterwards used for any other purpose than yeast inoculation. When a monkey was vaccinated in four places with arm-to-arm lymph, eight days after fermentation, only one insertion "took." The vesicle was remarkably retarded till the sixth day, Its effects. Fermentation and vaccination. Monkeys.

Fermentation and vaccination.

when the first appearance of the vesicle was observed. It increased with such rapidity that, on the eighth day it was typically Jennerian, perfectly circular, plump, and umbilicated, about the size of a sixpence. In a third monkey, although all four insertions of arm-to-arm lymph "took," they did not develop typical vesicles, but scabs only. From these experiments, therefore, I concluded that, though yeast has some protective power against vaccination, it is less powerful as a fermenter of the blood; but the same may be said, as is shown elsewhere, of pure cultivations of variola.

Monkeys.

Calves.

The experiments on fermentation of calves resulted in total failure to protect them, by yeast inoculation, from calf-to-calf vaccination. Dr Cory succeeded in producing typical vaccine vesicles in both the fermented calves which I sent to the National Vaccine Establishment to be tested. I used the lymph taken from these vesicles for the purpose of vaccinating six children, and admirable results were produced. I am unable to say whether fermentation produced a rise of temperature in calves, and I have not repeated the experiments to ascertain this, chiefly owing to the serious expense they entail. I attribute the failure to protect to the difficulty of grafting or planting the yeast in calves, so as to obtain a sufficient growth.

Fermentation and variolation.

With regard to protection by yeast inoculation from variolation, I have found that, although local vesicles can be produced with smallpox lymph in fermented monkeys, it is impossible to obtain a secondary constitu-

Monkeys.

tional eruption. The local effects were never more severe than ordinary vaccination, and the fever was mild, although arm-to-arm inoculation was practised. When an unprotected monkey is inoculated with confluent variolous lymph, the disease proceeds, locally, in exactly the same manner as an ordinary vaccination, and the secondary eruption of a few vesicles appears on the eleventh day. Indeed, the result is so mild that I am unable to see, from my experiments, how compulsory variolous inoculation with carefully selected lymph, after fermentation, can be more dangerous than vaccination. Good inoculators warmly defended the practice of variolous inoculation. Might not compulsory variolation be quite a safe proceeding? This question has been already answered in the negative by authorities in this country. I am informed, however, by Dr W. Aitken,[1] that the Chinese still prefer variolation to vaccination. Its value as an alternative proceeding in smallpox epidemics, where vaccine lymph cannot be obtained, is unquestionable, and in such circumstances I believe it could be used quite as safely and freely as vaccine lymph. In fact, the constitutional eruption may, apparently, be very much mitigated, or even entirely prevented, in proportion to the number of good vesicles produced locally. The mild result of variolous inoculation in unprotected monkeys impairs its value as a control experiment, and renders it impossible to say whether yeast inoculation is sufficient to prevent

Fermentation and variolation.

Monkeys.

[1] Tientsin, North China.

Fermentation and variolation.

the secondary eruption in artificially induced variola. But this result has been obtained indirectly. If we vaccinate fermented and variolated monkeys with vaccine lymph, I have found (Exps. 41, 42, 48, 49, 50) that they are, without exception, completely protected from the vaccine disease. On the other hand, a simply variolated monkey was found to be susceptible of the vaccine disease (Exp. 53), a good scab larger than that in Plate XVI. having been produced. It is convenient to note here that inoculation of white cultivation of variola does not produce any local vesicles; but an eruption during the first week. This variolous material is also imperfectly protective from the vaccine disease.

Results.

On carefully studying the results, combined in various ways, of successive fermentation, variolation, and vaccination of monkeys, I have come to the conclusion that yeast inoculation modifies the action of both the variolous and the vaccine poisons. Further, yeast inoculation can be safely carried out in the human subject, as is shown by Exp. 45; and I have also succeeded in producing, by it, a febrile condition in a monkey which had been both successfully variolated, and successfully vaccinated afterwards. Lastly, cultivations of the blood of monkeys appeared to show that yeast life may be carried on in the animal body in the form of minute spherical organisms, which are indistinguishable from micrococci.

CHAPTER X.

THE COMPOSITION AND ACTION OF NATURAL AND ARTIFICIAL VACCINE MATERIALS.

SECTION I.

Natural Vaccine Materials.

Natural vaccine materials.

Different vaccine materials can be distinguished by their naked-eye and microscopic appearances, and by their physiological action, as shown by the results of experience, and by experimental vaccination. Clear vaccine lymph, carefully taken from typical vesicles in healthy subjects, is regarded by the authorities of the National Vaccine Establishment as the most perfect material for vaccination that can be obtained. Opaque or opalescent lymph is regarded as an inferior or imperfect material for vaccination, on the ground that complaints used to be frequent because of its too energetic qualities. However, it is remarkable that, since perfectly clear lymph has been selected as standard vaccine lymph, the complaints have assumed a diametrically opposite tone, to the effect that it is too

Clear lymph.

Opaque lymph.

Natural vaccine materials.

inactive. This has led to the conjecture that some opalescent lymph which is now rejected, although supplied from the best vaccinating stations, may be a perfectly good material for vaccination, but the nature of the difference between them has not been satisfactorily made out.

Characters.

Vaccine lymph is said to be clear when it shows no opalescence by reflected, or obliquely transmitted, light, and when it is free from blood or other impurities. It is said to be opaque when it appears slightly milky, or opalescent, when tested in the same way. One of the great difficulties of vaccination is the propagation of a material which remains perfectly clear for a time after being stored. The successful propagation of clear vaccine lymph for storage depends on a chain of circumstances, one of the links of which may fail at any point, and vitiate the desired product. Either of the children, though not positively unhealthy, may be slightly below par; the vesicles may be irritated or injured during their progress; or the operation may have been unskilfully performed; or some intercurrent complaint may have come on; and any one of these is fatal to the production of clear vaccine material.

Difficulties in propagation.

Choice of operation.

It is found that vaccine lymph is most certain in its action when transferred directly from the arm of one child to that of another, good vesicles being easily produced by a single or double scratch thus, / //. If this same material be stored in a tube for even

half an hour it is found that the same result cannot be produced unless the operation is done thus ⁞⁞, and the lymph well rubbed in. This explains why many vaccinators fail with clear lymph, especially if they are in the habit of vaccinating from arm-to-arm. The material has changed in potency, and a more severe operation is required for its successful insertion. When a cover-glass preparation of clear lymph is examined microscopically, it shows minute isolated spherical organisms, about ·15 μ in diameter. This is the true bacteric form of the perfect vaccine material, which does not contain any larger organisms. When the lymph is dried, these organisms, which I regard as the spores of micrococci, are preserved from developmental changes, and the material undergoes no other change in quality than is common to stored lymph generally. This clear vaccine lymph gives the most perfect local results, and I define it as pure liquor sanguinis, with the spores of micrococci in suspension, which are pre-eminently contagious (see Plate VIII.).

Natu vaccine materials.

Composition and action of clear vaccine lymph.

Clear variolous lymph has the same composition, and it is probably similar in action, but for obvious reasons I have no personal experience of its action in the human subject. This, however, is furnished by the experience of the old inoculators, some of whom (*e.g.*, Baron Dimsdale) have recorded their opinion as to its efficacy and safety (see Plate XI.).

Variolous lymph.

Opaque vaccine lymph, unless originally clear and

Natural vaccine materials.

Opaque vaccine lymph.

carefully stored in well-filled sterilised tubes, from which the air has been expelled during the sealing process, must be regarded as an imperfect material for vaccination. It is generally produced by children who are below par, from vesicles surrounded by areola, or which have been injured or irritated. When such lymph is stored the opacity gradually increases, even in sterilised tubes; and even clear lymph gradually becomes opalescent. The opacity is observed also at the moment of its exudation from the vesicle, and opacity of clear lymph is not prevented, even when stored in sterilised tubes under shelter of carbolic spray. After observation of thousands of tubes of vaccine lymph, and some hundreds of sterilised and commercial vaccine tubes, containing sterile and non-sterile fluids, used to imitate vaccine lymph and the conditions under which it may be stored, I have come to the conclusion that it is impossible to prevent the occurrence of opacity in vaccine lymph stored in the fluid state. Tyndall and Pasteur have shown that only sterile fluids remain clear when stored, even when access of impure air is prevented. Fluids which contain germs or spores of any kind become turbid from the growth of these spores. I am indebted to Professor Chiene for the suggestion that the cause of opacity of lymph is a "germ," but my experiments appear to demonstrate beyond doubt that the "germ" which grows in vaccine lymph, and produces its opacity, is the "germ" of vaccinia itself. The sudden

Cause of opacity.

Natural vaccine materials.

appearance of opacity within the vesicle; its gradual increase in lymph, which is clear when stored; and the proved non-sterility of vaccine lymph; all point irresistibly to the conclusion that the organism which grows and produces the opacity, is an intrinsic constituent of the lymph, and not an accidental contamination from without.

Composition and action of opaque vaccine lymph.

When opaque vaccine lymph is examined microscopically we find that it contains larger organisms, ·5μ to 1μ, no longer isolated, but in rows, masses, and sarcina-forms, and there are a few torula or yeast forms ·2μ in diameter (see Plate IX.). Cohn is unable to say whether these are genetically connected with the isolated spheres in clear lymph; but if they are not, where do they come from? These larger organisms do not exist in fresh clear lymph; and I can come to no other conclusion than that they are developed directly from the spores existing in it. The definition of opaque lymph, then, is this, that it is a natural cultivation of the spores in clear lymph.

Experience teaches that opaque lymph is an imperfect vaccine material. It is more energetic locally than clear lymph, and in former times eruptions were frequently produced by its use, especially when the lymph was taken too late. I regard, therefore, cultivations of spores as a vaccine material which is more powerful in action than clear lymph, the local effect being modified, and the constitutional effect being more severe.

Natural vaccine materials.

Opaque variolous lymph.

Opaque variolous lymph may be regarded in the same way as a cultivation of the spores in clear variolous lymph, but its action in producing secondary eruptions when inoculated is more powerful, as I gather from the old records of smallpox inoculation. It produces a more severe, and undoubtedly infectious, disease when inoculated, and it should not be used under any circumstances. I have not examined calf-lymph microscopically, and can only therefore refer to the admirable work of Pohl-Pincus on Vaccination for details of its composition in the fresh condition. He finds that the micrococci are isolated, and 1 μ in diameter. They do not occur in balls or groups, but fresh lymph always contains them.

Calf lymph.

Pohl-Pincus.

Fresh.

Calf-to-child vaccination is as successful as arm-to-arm vaccination with human lymph, the results of Dr Cory's practice at the National Vaccine Establishment being most satisfactory. He first charges a lancet with the material, and makes five insertions by five separate punctures (twenty-five in all), into which the lymph is well rubbed while the skin is kept on the stretch to prevent bleeding. Vaccination with stored calf-lymph, supplied by Dr Warlomont, requires to be done very carefully to obtain successful results. Scarification, thus  , is performed with an ordinary sewing-needle in as many places as desired, and the lymph is well rubbed in. Unless the lymph is rubbed into the scratches there is a danger of the vesicles being retarded and abortive. This material is convenient when arm-

Stored.

to-arm lymph cannot be obtained, but not unfrequently secondary papules are observed during the second week. The local results are perfect. Natural vaccine materials.

Yeast-forms are found in both human and calf lymph when stored. Yeast-forms.

SECTION II.

Artificial Vaccine Materials.

In order to obtain a material for vaccination, reliable under all circumstances, great attention has been paid, not only to its propagation in man and animals, but also to its storage. Dr Husband's method of preserving vaccine lymph fluid and active in capillary tubes, well filled and hermetically sealed, has been adopted by the National Vaccine Establishment, which insists that the material shall be clear, in which proviso the whole difficulty lies. A non-sterile fluid stored in such tubes invariably becomes opalescent after a time. Dr Seaton regards dried lymph as an inferior material to fluid lymph, but if my explanation of the cause of opacity be correct, this cannot be the case. It is only a little more difficult to make it take, and this can be overcome by varying the method of insertion. If a sufficient surface be abraded, and the lymph carefully rubbed in afterwards, a very small quantity only of dried lymph is sufficient to produce a good vesicle. If dried lymph fails to take, the fault lies with the operator, not with the material. It has been supposed that quantity as Artificial vaccine materials. Storage.

Artificial vaccine materials.

well as quality of the material is necessary for success; but in my opinion, if the quality of the material is perfect, the quantity of the operation is of more importance.

Müller's method of multiplying lymph.

Müller was the first to discover that vaccine lymph, when mixed in certain preparations with glycerine and distilled water, does not lose its active properties (Ziemssen's *Encyclopædia*). He mixes one part of vaccine lymph with two parts of glycerine and two parts of distilled water, and finds that this material produces good vaccine vesicles. When clear lymph is employed, and when the mixture is declared, I can see no objection to this proceeding. It is, however, sometimes used fraudulently as clear lymph. The treatment of opaque lymph in this way is objectionable, but is easily recognised by its optical properties and a regular white streak of opacity in the tube. It also takes longer to dry on the arm. It is unlikely that further development of opacity takes place in lymph treated in this way, this being prevented by the antiseptic property of the glycerine. The material cannot therefore, be looked upon as a cultivation of lymph, as there is probably no increase in the number and size of the organisms such as takes place in opaque lymph.

Quist's method of cultivation.

Quist[1] (*Berliner Klinische Wochenschrift*, No. 52) has succeeded in artificially multiplying lymph in a fluid medium composed of equal parts of blood-serum, glycerine, and distilled water, which is rendered alkaline

[1] Trans. by Dr Francis Troup, *Edin. Med. Jour.*, 1884, p. 1052.

by the addition of $\frac{1}{300}$th part of carbonate of potash. The liquid is inoculated, after being sterilised by heating it to 60° C. for an hour and a half on three successive days, by a minute piece of sterilised sponge soaked in clear lymph, or by a piece of crust from a vesicle which has been washed in distilled water and then carefully dried. The growth on the surface consists of very minute scales, which are inoculable after a few days. Below the surface a finely pulverulent sediment falls to the bottom. The micrococci are very minute, the scales being composed of swarms of them. When preserved in capillary tubes the fluid retains its power for weeks. It protected from a second vaccination in one case. Quist considers that the propagation of lymph outside the animal body depends on two things, the presence of oxygen and a suitable cultivation medium. Apparently he has still to show whether the fluid retains its active properties after any number of such generations in his cultivating fluids, and how long it can remain active and unchanged when stored. I have no doubt that the micrococci he describes correspond very closely indeed to the spores in clear lymph, but I have not yet repeated his experiments successfully. His observations, however, prove the value of Müller's discovery, and his nutrient fluid can apparently produce the bacteric form associated with true human vaccine material. I agree with Quist in thinking that pure cultivations of lymph require free access of oxygen.

Artificial vaccine materials.

Quist's method of cultivation.

Artificial vaccine materials.

Orange and brown vaccine.

The nearest approaches to the micrococci grown in Quist's fluid which I have seen in solid media are the orange and brown vaccine. The orange growth occurs in Koch's nutrient gelatine and the brown in agar agar. Both (Plates I., II.) show swarms of micrococci ·3 μ to ·4 μ in diameter. They are thus larger than the spores in clear lymph. Inoculation with orange vaccine produced no local effect in a calf, but in a guinea-pig a local scab was produced, similar to that produced by yeast inoculation in monkeys. Pure orange cultivations (Plate VII.) show slightly larger organisms, ·5 μ to 1 μ, as if the vegetative growth was more active. I cannot distinguish these from pure white vaccine or variolous cultivations, either by size or arrangement (compare Plates VII., X.). The conclusion from the guinea-pig experiment is that orange vaccine is more active locally than either white or yellow vaccine. No experiments were made with brown vaccine, but I consider it very nearly the same as the orange growth.

Yellow vaccine.

Yellow vaccine is characterised by its regular arrangement of the units in dumb-bells forming fours which resemble sarcinæ. The sarcina-forms may be simple, when the units are very minute, ·2 μ in diameter; or multiple, when the apparent units are larger, 1 μ in diameter. Its action in a calf and guinea-pig was not local, but probably constitutional. In my opinion, it is a true vaccine, being derived from a source which produced a pure yellow growth (see Plates IV., V.).

White vaccine occurs as dumb-bells and single

Artificial vaccine materials.

White vaccine.

micrococci, in diameter ·5 μ, which are the true units, and these may be arranged in fours, which differ from true sarcina-forms by their lozenge shape. They also form chains when the dumb-bells are placed end to end. I have also seen it, apparently, as an early stage of the yellow vaccine. Pure cultivations show larger units, 1 μ in diameter, and the dumb-bell arrangement is more distinct (see Plates III., VI., VII.). It produces no local effect either in a calf or a guinea-pig, but all three cultivations, white, yellow, and orange, being derived from different sources, must be regarded as having assisted in producing a constitutional eruption in a calf.

Their effects.

When human vaccine lymph is used for inoculating cows or calves, the production of vesicles is limited entirely to the points of inoculation (Seaton's *Handbook*, p. 16). The discovery, therefore, that neither white, yellow, nor orange vaccines produced local vesicles, but that all three combined produced a general eruption, led me to the conclusion that modified vaccine materials, produced by growing lymph in solid media, were unsuitable for vaccination purposes; because, if vaccine lymph be really modified variolous lymph, then modified vaccine materials were further modifications of a modification, and success in this direction was impossible. It appeared more probable that variolous lymph might be easily modified by artificial cultivation, otherwise than in animals, and that this cultivation

Artificial vaccine materials.

of variola would be a true modification of the specific virus, having properties of a much milder character than the original. This led me to undertake the cultivation of smallpox.

White variola.

The only artificial material derived from smallpox with which I have experimented is white variola, the mode of growth and histological characters of which are, apparently, identical with those of white vaccine. Pure cultivations occur chiefly as dumb-bells, the smallest units of which measure ·4 μ in diameter, and the largest ·8 μ to 1 μ in diameter. Its action in monkeys is exactly the same as that of vaccine cultivations in a calf; no local vesicles, but a constitutional eruption, being produced when it is inoculated. Cultivations from these vesicles produced a white growth, consisting of organisms, identical in character and mode of growth with that which furnished the material for inoculation. Similar, if not identical, organisms are also formed in opaque variolous lymph, which also produced an eruption in a calf, without local vesicles (see Plate XI.).

Its effects.

The natural variolous material, however, differs remarkably from the artificial. It produces local vesicles exactly like vaccine vesicles, and the first appearance of the secondary eruption does not take place till the eleventh day (compare Hennen). (Plate XXIII.). White variola produces no local vesicles, and the first appearance of eruption is on the fifth day from inoculation (Exps. 9, 12). The time, therefore, of incubation

of variolous cultivations is about half that of the natural virus. The whole course of the disease is milder than that of smallpox inoculation in unprotected monkeys, but neither material is completely protective against the action of the vaccine virus (Exps. 11, 53). If, however, a monkey is first inoculated with dried yeast, and then successfully inoculated with natural variolous material, the vaccine virus produces no effect (Exps. 41, 42, 48, 49, 50). I have not been able to obtain pure cultivations of yellow and orange growths from variola, but the table of cultivations shows white, yellow, and orange precipitates, and these are interesting as showing the possibility of obtaining growths of variola from different sources, which agree in character with those obtained from different vaccine vesicles. As already indicated, I regard these different vaccine and variolous materials as developmental conditions, growth-forms, or variations of a single micro-organism, and not as different species, having no relation to each other.

Artificial vaccine materials.

White variola.

Yellow and orange variola.

The presence of yeast-forms in acid lymph suggested that a yeast ferment, which had become acclimatised in man, might be the original cause of smallpox, and might explain the universal occurrence of the disease in unprotected persons. Although the experiments are still imperfect, I am of opinion that they clearly show that, when dried yeast is inoculated in monkeys, it has both a local and constitutional action, producing raised scabs, beneath which there is a production of

Original cause of smallpox.

Artificial vaccine materials.

Dried yeast.

lymph, from which I have obtained cultivations of micrococci. It is unfortunate that no cover-glass preparations were made and dried, as these would have shown the bacteric forms from which the cultivations developed. The acclimatisation should also have been carried further by arm-to-arm inoculation with this lymph, to ascertain whether the local effect became increased.

Its effects.

I have no doubt, however, as to the occurrence of febricula in monkeys after yeast inoculation, and my conclusion from the experiments is that yeast has the power of growing in the tissues when introduced into the body in a potential form, such as dried yeast, which is a material not easily destroyed, as is shown by the smugglers' method of storing it. When inoculated it is absorbed, and compelled to grow in the absence of free oxygen, and it requires to abstract this, probably from the blood-cells. In this way it may act as a true ferment within the body, and set up a febrile condition.

Comparison of vaccine materials.

On comparing, therefore, the natural and artificial vaccine materials, we see that the bacteric form which most certainly produces local vesicles is the spore in clear lymph, and that the larger organisms in opaque lymph have been proved, by experience of such material, to act locally more violently, and even to produce eruptions. Many instances of this are to be found in the older works on vaccination and inoculation. We must therefore regard opaque lymph as more energetic than clear lymph.

Comparison of vaccine materials.

Artificial cultivations in fluids, as has been shown by Quist, if grown in shallow vessels, retain the bacteric form of clear lymph on the surface, but for how long is uncertain. His cultivations act locally, but they are not pure bacteriologically. No doubt, however, they are practically pure, just as a good vaccination, producing typical vesicles without areola, is clinically a pure cultivation of the vaccine virus. Artificial cultivations of vaccinia and variola in solid media have no local action whatever, the organisms being immediately absorbed, and producing secondary foci in half the time required by the natural lymph.

Animal vaccination.

I prefer to leave the discussion of animal vaccination to those having large experience of it, and content myself with merely expressing the opinion that it is a valuable alternative proceeding when arm-to-arm vaccination cannot be carried out. The material is different from clear human vaccine lymph, the organisms in the calf, according to Pohl-Pincus, measuring 1 μ in diameter. Theoretically, therefore, it is more powerful, but its energetic action is apparently modified by the change in its environment when transferred to children.

CHAPTER XI.

IMMUNITY AND ATTENUATION OF VIRUS.

SECTION I.

Immunity.

Immunity. Its production.

SIMON says that "to the present time (1871) it remains one of the most interesting and least explained facts in pathology, that the specific contagion or ferment of smallpox, so uncontrollable in its operations when it enters a man in the ordinary way of his breathing an infected atmosphere, becomes, for the most part, disarmed of its virulence, when it is artificially introduced to the system through a puncture of the skin." He defines cowpox as "the smallpox of the cow," and adopts Jenner's view, that "smallpox and cowpox are modifications of the same distemper, so that, in employing vaccine lymph, we only make use of means to impregnate the constitution with the disease in its mildest, instead of propagating it in its virulent and contagious form, as is done when smallpox is inoculated."

I have tried to show that the vaccine and variolous contagia are not dissolved in the lymph, but are suspended in it, in the form of very minute particles, which

I look upon, from their size, as spores of micrococci. Standard vaccine lymph is alkaline, and contains spores in suspension, and these must be regarded, when judged by the effects of their pure cultivation in the animal body, as the true vaccine contagium. Good vaccinators vaccinate with spores. Opaque vaccine lymph represents developmental conditions of the spores in clear lymph; and, when judged in the same way by its effects, the conclusion has been arrived at, by authorities on the subject, that it is an imperfect or inferior material for vaccination. Good vaccinators, accordingly, do not vaccinate with cultivations of spores. But this development of organisms in lymph produces acidity, not only in the tubes, but within the vesicles, so that it may be fairly deduced that the contagium of vaccinia is a ferment producing acidity of alkaline fluids within the body. This conclusion is strengthened by the appearance of yeast forms in opaque lymph, distinguishable by their size from other cells of animal origin. Vaccination is, in my opinion, the artificial growth of a ferment on the human skin, the products of which are absorbed, and grow in the blood until the whole of the blood is affected, after which it is impossible, for a time at least, to repeat the process in the same person, who is then said to be protected.

Immunity.

True material.

Imperfect material.

Nature of vaccination.

Various theories have been advanced to explain immunity, that of a contagium vivum being now generally accepted. It is said that the growth of the virus uses up or exhausts the whole of the material

Immunity. Leading theories.

necessary for its subsistence, and it then dies a lingering death from starvation—the Exhaustion Theory.[1] Another theory is that the virus secretes or excretes another poison which is fatal to its present and future existence, but which is tolerated by the human system for a long period afterwards—the Ptomaïne or Antidote Theory.[1] Lastly, it is supposed that, in abstracting from the blood or tissue-cells material for it subsistence, the cells themselves are transferred, and acquire the same power as that possessed by the virus—the Theory of Metabolism.[2]

Considerable light is thrown upon these leading theories by what is known to take place during the process of malting, and during alcoholic fermentation, about which I am indebted to Mr Duncan M'Glashan for the following interesting information. In malting, a heap of barley is well moistened with water, and then kept at a certain temperature until the grain commences to sprout. The bud, however, is not allowed to burst through the husk of the grain. Before this takes place, the barley is spread out in a thin layer in the barn and dried, the product being malt. It is well known, from chemical research, that during this process the starch of the grain is converted by the action of diastase into dextrin and grape-sugar. It is, not, however, generally known that malt has a peculiar power of its own. When distillers are preparing a saccharine solution for distilling purposes, they find

Malting.

[1] Klebs. [2] Grawitz.

that it is unnecessary to employ malt entirely. They mix one part of malt and nine parts of unmalted barley in the "backs" with a large quantity of warm water, which is kept constantly stirred for some time, at the end of which it is found that the whole of the starch in the unmalted barley has become converted into grape-sugar. Practically, it is found that malt can convert the starch contained in nine times its weight of barley into grape-sugar. Its action is the same as the original action of the diastase, and the fact suggests the inquiry whether diastase is not really an organised vital ferment, capable of indefinite multiplication. The essence of the process is that the mixture is kept constantly stirred at a warm temperature, and the product is a solution of grape-sugar, which is used for fermentation.

Immunity. Leading theories. Malting.

If a quantity of yeast is now sown in this saccharine solution, which is alkaline, it grows very rapidly, and produces alcohol and carbonic acid. In practice, however, it is found that fermentation stops, either when all the sugar is converted into alcohol, or when there is more than a certain percentage of alcohol in the solution. In either case, if the solution is left to itself, the material become acid, and this acidity takes place somewhat suddenly. It does not appear to be necessary, in my opinion, to invoke the aid of another bacterium to explain this acidity, as is usually done. Before acidity takes place, however, if either the sugar is exhausted, or if the solution contains too large a percentage of alcohol, the yeast cannot continue to multiply,

Fermentation.

Immunity. Leading theories. Fermentation.

and the addition of any quantity of fresh yeast fails to excite further fermentation. Before this can take place, the alcohol must be removed by distillation, or more sugar must be added to suit the circumstances.

The process of fermentation, therefore, is strictly analogous to the multiplication of morbid poisons in the blood, and affords a satisfactory explanation of the so-called Exhaustion, and Antidote or Ptomaïne Theories of protection. It fails, however, to explain the last-mentioned theory, because the nourishing material does not possess any intrinsic vital property similar to that possessed by the blood. The saccharine solution is entirely passive; the blood is actively vital; and experience shows that it becomes changed in a remarkable manner by the action of the poisons of infective diseases. The process of malting appears to afford a satisfactory explanation of the metabolism of the blood, produced by infective poisons. The diastase may be taken as analogous to the vaccine material, and it multiplies and exhausts the starch till it is all transformed into sugar, when its action is stopped by heat. This is the stage of exhaustion produced artificially. If, now, fresh unmalted barley be mixed with malt and constantly stirred, the same process continues until the stage of exhaustion is again reached, and apparently this might be continued indefinitely so long as fresh material was added. The original quantity of malt may be looked upon as analogous to vaccinated blood, and the unmalted barley as analogous to unvaccinated blood.

Explanation of theories of immunity.

Immunity. Explanation of theories.

When we vaccinate infants, we introduce into a fresh or unexhausted nourishing soil a minute quantity of a ferment, which possesses the power of multiplying within the body, both locally and generally. During this process a febrile condition is produced, presumably by fermentation of the blood, and finally the stage of exhaustion is reached. This is shown by the failure to infect the infant a second time with vaccine material. In this state the blood may fairly be compared to malt, and its action on fresh material is probably similar. Vaccinated blood, stirred up by the circulation with fresh material, bears the same relation to it that malt does to unmalted barley, and experience shows that it possesses the same power of communicating its properties to fresh material. In the case of malt, it is assumed that the transformation is produced by a chemical, not a vital action; but I confess that this view is to me an unsatisfactory one, as I incline to the belief that the process may depend on the action of a vital ferment. The same difficulty does not exist in accepting the view that the whole mass of vaccinated blood possesses the properties of vital ferments to unexhausted new material. It is, in fact, the antidote against the recurrence of the disease through the continued exercise of its altered vital power. Experience shows, however, that in course of time this metabolic power becomes weaker, and individuals gradually acquire a renewed susceptibility to the vaccine disease. This change is best explained by the view that the

Immunity. Explanation of theories.

constant waste and renewal, which the blood undergoes, at last reduces its metabolic power to the lowest ebb, and then the period of protection comes to an end. The blood returns in great measure to its first estate, when it is analogous to a saccharine solution, and favourable to a second growth of either vaccine or variolous virus. The wonder is, not that vaccination wears out, but that its protective power endures so long, in many cases for a lifetime. For the full explanation, therefore, of the immunity afforded by vaccination, it is necessary to combine the Exhaustion, Ptomaïne, and Metabolism Theories.

SECTION II.

Attenuation of Virus.

Attenuation of virus.

The mitigation or attenuation of the virus, quoted from Mr Simon's paper at the beginning of this chapter, is a subject of great interest and importance, and, in my opinion, like immunity, depends on various factors, any one of which is insufficient for its explanation. Pasteur insists that in anthrax it depends on the temperature at which the organism grows, and on the action of oxygen. Chauveau thinks that attenuation of anthrax poison is not produced by the oxygen of the air, but by spore-production. Greenfield thinks that attenuation is produced by the antiseptic action of a chemical

substance produced either by the organisms themselves or by tissue-metamorphosis. All three views are, in my opinion, perfectly reconcilable, for spore-formation can probably only take place in presence of oxygen, either free or in combination; and Chauveau has shown that spores are attenuated by heat; while Greenfield's theory possibly resolves itself into metabolism produced by their action.

Attenuation of virus. Pasteur. Chauveau. Greenfield.

It is dangerous, however, in the study of micro-organisms, to conclude that what is true of one organism is true of another, and it is often difficult to resist the temptation to draw general conclusions from inadequate data. It cannot be too strongly affirmed, as Koch has already done, that every infective disease must be investigated by itself, but it is certainly very satisfactory to have an opinion, arrived at independently, "that the true vaccine material for infective diseases is to be found in the spores of the micro-organisms which are their exciting causes," corroborated by such a distinguished authority on vaccination as Chauveau. This is certainly the key to the line of inquiry to be followed in the case of every infective disease. With the knowledge of the conditions under which micro-organisms form spores, and spores only, it will probably be possible to modify their action by methods of artificial cultivation. I am inclined to attribute Pasteur's success, in attenuating the virus of chicken-cholera and hydrophobia, to the fact that he has obtained them in spore-form. Probably,

Author's view.

Attenuation of virus.

also, the secret of Ferran's success in cholera inoculation can be explained in the same way; and the tendency to scepticism on the subject should, in my opinion, be checked until the matter is set at rest by further scientific investigation.

CHAPTER XII.

CLASSIFICATION OF BACTERIA.

For pathological purposes the cleft-fungi or Bacteria are usually conveniently separated from the mould-fungi and the yeast-fungi, but botanical authorities differ in opinion as to the correctness of this division. Thus, Brefeld regards yeasts as probably mere low forms of the moulds or filamentous fungi; while Naegeli and Sachs class them with the Bacteria as Protophyta. The yeasts, therefore, apparently occupy an intermediate position between the mould-fungi and the cleft-fungi, and all three are placed by Sachs among the Thallophytes, which now include both Algæ and Fungi. Within the last few years the classification of the Thallophytes has been modified since the discovery of the reproductive organs, and in many cases of the entire life history of the various forms, and the observations of various botanists, such as Cohn, Brefeld, De Bary, Hueppe, Flügge, and Zopf, have contributed largely to this result.

Classification of bacteria.

Views of botanists.
Naegeli.
Sachs.
Cohn.
Brefeld.
De Bary.
Hueppe.
Flügge.
Zopf.

Since the publication of Cohn's researches on Bacteria in 1872, the fission-fungi have undergone the most minute investigation, not only by botanists, but

Classification of bacteria.

by pathologists and other scientific observers; and there has been such an accumulation of literature on the subject that it has been thought necessary to revise and extend Cohn's classification, so as to include the new discoveries. Hueppe[1] and Baumgarten,[2] who follow Cohn's classification, give a good account of these modifications. The new classifications, *e.g.*, those of Zopf and Flügge, are based, like that of Cohn, chiefly on morphological and physiological characters; but, as they are stated to be only provisional, I prefer to employ Cohn's original classification, which is still substantially accurate. Cohn believes that the bacteria are capable of exact specific classification. Owing to their minuteness, it is in some cases impossible to make out any structural differences, and in these circumstances he takes into account the size and shape of the cells, and the appearances of the colonies, as the criteria of specific differences. The shape of bacteria is a more reliable character than size, which he considers may vary within certain limits. He has not hitherto been able to discover differences in the mode of reproduction of these very minute bacteric forms, so that his genera and species are characterised by the characters of their vegetative cell-forms, and not by their mode of reproduction. He considers his method defective in this respect, as it is probable that some of such

Baumgarten.

Views of botanists.

Cohn.

Hueppe.

Flügge.

Zopf.

[1] *Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten*, 1886.

[2] *Lehrbuch der Path. Mykologie*, 1886.

genera and species really represent different developmental stages of one and the same fungus. The whole question of classification must be regarded at present as in a state of transition. Classification of bacteria.

Cohn divides the Bacteria into four well-known groups—Sphærobacteria, Microbacteria, Desmobacteria, and Spirobacteria; but we have only to do with his first group, the Sphærobacteria, comprising one genus, the Micrococci, which he maintains is constant in form. The bacterial spherules, described by other observers as growing, *e.g.*, into rods, would be more correctly described as spores of bacilli, and should not be called micrococci. Cohn provisionally distinguishes micrococci into species by their physiological characters; such as production of different colouring matters; their effects on the soil or liquid in which they grow; and their power of producing disease; and he describes three such physiological species—(1) Chromogenic; (2) Zymogenic; (3) Pathogenic. Views of botanists. Cohn. Ziegler. Zopf.

Ziegler mentions another genus of Sphærobacteria, the Sarcina, the species of which are distinguished according to the size of the cells; and he states that it is not yet certain whether micrococci produce spores.

Zopf includes the Sphærobacteria of Cohn in a group which he calls Coccaceæ, and divides into five genera; Streptococcus, or Chain-cocci; Merismopedia, or Plate-cocci; Sarcina, or Packet-cocci; Micrococcus, Swarm or cocci; and Ascococcus, or Bladder-cocci. De Bary objects to mere form-nomenclature, of which the

Classification of bacteria.

above classification of Zopf is a good example, and indicates his strong opinion that nomenclature should depend on the capability of micro-organisms for further development. On this ground, I think, Zopf's nomenclature open to objection, as the name "coccus" is common to developmental conditions of bacteria belonging to his other three main groups, and this is apt to lead to confusion. Cohn's nomenclature is much simpler and more elastic as regards the inclusion of growth-forms.

From the above it is easily seen that botanists are keenly alive to the defects in their classifications of bacteria, and one of them (Brefeld) even goes the length of demanding cultivation from spore to spore, before deciding as to specific differences between different forms of bacteria. In mentioning this demand of Brefeld's, Koch dismisses it as impossible to fulfil at present, as regards the pathogenic bacteria, although he admits that it is theoretically correct.

Views of botanists.
Zopf.
Cohn.
Brefeld.

In Koch's opinion, founded upon his researches on traumatic infective diseases, a distinct bacteric form corresponds to each infective disease at a certain stage. When this form is found for any disease, its cultivation from spore to spore may be undertaken. Koch further maintains that the same bacterie form is found in each disease, however often the disease be transmitted from one person or animal to another. He also calls attention to the difficulty attending the recognition of specific forms of bacteria, when such forms are very

minute and indefinite. He considers that when infective materials, with the nature of which we are not yet precisely acquainted, for example, variolous and vaccine matter, remain active in a dry state for a long time, even for several years, these cases depend on the existence of a real resting-stage of the organism. While, therefore, Koch insists on constancy of the bacteric form in a certain stage of each infective disease, he frequently, in his writings, shows that he tacitly admits the probable existence of growth-forms different from such a form. For example, if the bacteric form were proved to be a spore; by histological examination, successful inoculation of the disease, and the invariable discovery of the same bacteric form or spore in the diseased tissues after any number of inoculations; Koch would regard it as proved that it is the proximate cause of the disease. If pure cultivations of these were made, he would probably regard these as vegetative conditions or growth-forms. According to the present mode of classification these growth-forms would be regarded as different species, especially if it were to be found that they did not produce the disease when inoculated. In my opinion, supposing the true contagion to be a spore, and the growth-forms to be true vegetative conditions of the spore, failure to reproduce the disease in the same form does not necessarily indicate total failure of the inoculation. The spore may exert an evident local action, while the vegetative form may act constitutionally in a manner

Classification of bacteria.

Bacteric forms.

Koch's opinions.

Classification of bacteria.

difficult to discover and define, so that failure to produce a disease by inoculation may depend on a difference in physiological action between the embryonic and the more mature form of the organism. I should not, however, regard this as true mutation of a species, as the pathogenic properties of the organism would still be exerted, but would differ only in degree of

Mutability.

potency. The admission of developmental forms of a micrococcus, therefore, is not equivalent to the admission of mutability of species of micrococci. The physiological action of such developmental forms may differ in many cases, within certain limits, from that of the bacteric form which is the true cause of the disease.

Resting-stage.

Cunningham.

Dallinger.

The researches of Dr Douglas Cunningham upon the micro-organisms found in the intestinal canal, clearly show that they have an active and a resting stage. In a suitable medium the "zoospores" multiply till the material for their growth is exhausted, and the micro-organisms cannot develop further. When transferred to an alkaline medium they at once become active. The mode of reproduction of a "minute septic organism" has been well described by the Rev. W. H. Dallinger, in a remarkable paper communicated to the Royal Society of London in 1878 by Professor Huxley. He finds that the bacterium grows to an ovoid body of very delicate nature, which bursts and gradually collapses, scattering very minute spores. By continuous observation, he has seen the spore grow again into the

ovoid body, which then, after a short resting stage, burst as before. Similar observations, with regard to the life history of bacteria, have been made by Professor Cossar Ewart; and Mr P. Geddes points out that a resting stage occurs in many instances in the cycle of cell life.

Classification of bacteria.
Resting-stage.
Ewart.
Geddes.

It thus appears that size, arrangement, and physiological action, taken separately, are unreliable guides to the determination of specific bacteria, and that the study of their mode of reproduction and development is required in each case. Zopf and De Bary both maintain that in the bacteria such developmental conditions or growth-forms often occur, and there is no doubt that the recognition of this fact must give a great impulse to the further investigation of the bacteria. In the search for specific forms the growth-forms have been overlooked, or have been regarded as impurities, and thus the explanation of observed facts and experiments has been rendered fallacious. I do not maintain for a moment that impurities never occur, or even that they do not frequently occur, but these are in cases, such as tubercle and septic diseases, where pure cultivations are very difficult to obtain at first. With a material like vaccine lymph, and reasonable skill, it is comparatively easy to obtain pure cultivations. The difficulty appears to me to be to explain them after you have got them.

Reproduction and development.
Zopf.
De Bary.

CHAPTER XIII.

LIFE HISTORY OF THE VACCINE CONTAGIUM.

TABLE *showing the Histological Appearances of the Vaccine Organism in Solid and Fluid Media.*

Life History of vaccine contagium.

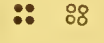
No.	Med.	Material.	Forms.	Corresponding Forms.
1	Fluid.	Clear lymph.		Burdon-Sanderson's 'microzyme.' Cohn's spheroidal corpuscle.
2	,,	,,		Cohn's simple corpuscle or M. Vaccinæ.
3	Solid.	White in jelly.		Cohn's 'pairs of corpuscles.'
4	,,	White in agar.		Cohn's 'groups' or 'clumps.'
5	,,	Yellow in jelly.		Cohn's 'groups resembling sarcinæ.'
6	,,	Yellow in agar.		Cohn's 'larger aggregations' or 'clumps.'
7	Fluid.	Opaque lymph.		Burdon-Sanderson's 'semifluid material' or 'oil-drops.' Cohn's "refractive cells resembling oil-drops.'
8	Solid.	Orange, 8th day.		Burdon-Sanderson's 'microzyme.' Cohn's 'minute spheroidal corpuscle.'

TABLE *showing Appearances of the Variolous Organism in Solid and Fluid Media (after Cohn).* Life history of vaccine contagium.

No.	Med.	Material.	Forms.	Corresponding Forms.
1	Fluid.	Clear lymph.		Cohn's minute sphere.
2	,,	,,		Cohn's simple corpuscle.
3	Solid.	White in jelly.		Cohn's pairs of corpnscles.
4	,,	,,		Cohn's 'groups' or 'clumps.'
5	Fluid.	Opaque lymph.		Cohn's 'groups resembling sarcinæ.''
6	,,	,,		Cohn's 'larger aggregations.'
7	,,	,,		Cohn's 'refractive cells resembling oil-drops.'
8				

Present state of knowledge.

The life history of the micro-organisms associated with variola and vaccinia, must be studied both within and without the animal economy. Pure cultivations of both diseases in the human subject have been obtained for the greater part of a century in each of them, by the employment of the natural virus for inoculation; but it is only within the last twenty years that any accurate knowledge of its composition has been obtained. Keber, Hallier, Weigert, Zürn, Cohn, Burdon-Sanderson, Klein, Pohl-Pincus, and Pfeifer, are the chief contributors to the literature of smallpox and vaccination from the bacteriological point of view. The tissues have been examined by Weigert, Klein, and Pohl-Pincus, while Cohn and Burdon-Sanderson

Life history of vaccine contagium.

have made exact researches into the nature of vaccine and variolous lymph. Dr Lionel Beale, another able worker in the same field, declares himself (1878) sceptical as to the occurrence of bacteria in lymph.

As the appearances in lymph are difficult to explain without the assistance of pure cultivations in solid media, I shall first summarise the latter. From different sources showing perfectly characteristic vesicles, clear vaccine lymph produced pure white, yellow, and orange growths in Koch's gelatine, which can be cultivated pure. Of these, accepting the view that vaccinia is merely modified variola, I consider the white growth to be typically specific, as the same form was found in pure variolous cultivations. Yellow and orange growths were also obtained from both variola and vaccinia, in each case from different sources. A brown vaccine growth was also obtained from agar agar. The naked-eye appearances, therefore, indicate that we have four, or at least, three, different bacteric forms in both vaccinia and variola, which can be purely cultivated. But as only one bacteric form corresponds to each infective disease, only one of these cultivations, if pure, should reproduce the vaccine and variolous vesicle at the point of inoculation. As a matter of fact, none of these materials does so, and we are forced to conclude that none of them is the true cause of vaccinia.

Appearances of vaccine cultivations.

Histological investigation of the different colonies only confirms this view, as the white, yellow, and

orange colonies show a different size and arrangement of their elementary units. When we examine clear vaccine lymph, these organisms cannot be found, although they are easily stained. Excluding contamination, for the sake of argument, it is found that a minute quantity of the same vaccine lymph, used for our cultivations, has produced in every case a vaccine vesicle; so that, if such lymph contains organisms which grow in Koch's gelatine, each white, yellow, and orange growth is an artificial imitation of the contents of a vaccine vesicle. If, on the other hand, contamination has occurred, a white, a yellow, or an orange organism has accidentally got into the lymph, either while in the vesicle, or as it issued from it. When it is remembered that all the cultivations were performed under the shelter of carbolic spray, this explanation is improbable. But even were it the case that this has happened, how is it that variolous and vaccine cultivations should show the same naked-eye characters, although they were made 400 miles apart? It is very unlikely that yellow and orange contamination of a white organism, should occur accidentally in every case. It is more probable that the clear lymph produced in each case a white, yellow, or orange growth, and that no contamination occurred. Just as one vaccine vesicle differs from another in different infants, so vaccine lymph from different sources may produce different coloured artificial growths.

Life history of vaccine contagium.

Explanation of appearances of vaccine cultivations.

The histological examination of cover-glass prepara-

Life history of vaccine contagium.

tions of fresh clear vaccine and variolous lymph shows a constant minute bacteric form or spore, distinguishable from the organisms in the cultivations by its size, numbers, arrangement, and action. It possesses powerful resistant properties, not being destroyed by other germs when preserved in the dry state.

The examination of stored vaccine lymph shows the same forms, but much larger, which coincide almost exactly with the organisms in the different cultivations in size and arrangement. If these are contaminations, they must come from without, but their presence is indicated by opacity of the lymph, and we know that this takes place within the vesicles. Cohn has seen them develop, so that without doubt growth of organisms takes place in opaque lymph; opacity of lymph also takes place universally; so that, if the growth is accidental, contamination of lymph invariably occurs during storage in tubes. But the organisms, if not a contamination, must pre-exist in the lymph; if opacity outside the body is due to growth of organisms, it must also be due to the same cause (excluding leucocytes, which are not found in it) within the vesicles; only it will be much more rapid, the conditions being more favourable. Opaque lymph must therefore be regarded both inside and outside the vesicles as a natural cultivation of the spores in clear vaccine and variolous lymph; and white, yellow, and orange vaccine and variolous cultivations must similarly be looked upon as artificial growth-forms in Koch's gelatine.

Histological investigation.

Life history of vaccine contagium.

It appears, therefore, from histological investigation, that the bacteric forms which occur in vaccine and variolous cultivations, and in clear and opaque vaccine and variolous lymph vary considerably; as may be seen from a careful study of the plates. The only thing that is constant is the shape or form of the organisms, and this is spherical. The arrangement of the units varies in the different materials; and there is also variation in colour. Thus, in clear lymph the units are isolated (Plates VIII., IX.). In opaque lymph we find several varieties of arrangement; and although pure white and orange vaccine and variolous cultivations cannot be distinguished from each other, yet primary pure cultivations, as shown by their colour, differ also in arrangement. Cultivations of lymph, taken from different sources, present the appearances described as characteristic of Zopf's five genera of Coccaceæ; and the appearances may be varied at pleasure by the mode of preparation. The size varies from ·15 μ to ·5 μ, 1 μ to 1·2 μ, 2 μ to 5 μ, the two largest sizes being reached by the peculiar bodies resembling yeast. The point of greatest interest is that the vaccine materials described differ in their physiological action. While the materials containing very minute organisms produce local pocks, those containing or composed entirely of larger organisms produce either an imperfect local effect, or no local effect, but an eruption in some other part; so that, in the case of the vaccine and variolous contagium, it becomes possible to distinguish

Histological investigation.

Life history of vaccine contagium.

between the action of the embryonic form of the organism, and the action of what must be regarded as developmental conditions or growth-forms of the organism. The action of the spore, which in this case is the true contagium, is local, while that of the mature organism, which is an imperfect material in opaque lymph and cultivations, is constitutional, not local. Not only so, but a distinction can also be drawn between the action of opaque lymph, which contains a comparatively small quantity of the contagium in this stage of development, and that of vaccine and variolous cultivations in solid media. For, while

Physiological action of materials.

opaque lymph generally produces a local vesicle, and not infrequently a constitutional eruption, which varies much in severity, the cultivations not only produce no local effect, but the period of incubation is much shorter, the general or secondary eruption appearing on the fifth day after inoculation. This shows that none of the effects produced is due to the original material of primary cultivations, because, if so, a local effect would have been produced. In addition, the modification of the variolous virus is effected by one generation of artificial cultivation, as in the cow. If a primary cultivation of variola be used for inoculation, the effect on a monkey is evident; but if a pure cultivation is used, the effect is milder (two vesicles), so that probably by a third generation no evident effect would be produced, because, with each pure cultivation, the modification or attenuation is being increased.

Life history of vaccine contagium.

The absence of local effect, when both vaccine and variolous cultivations were inoculated, led me to conclude that an artificial vaccine material, identical in potency with standard vaccine lymph, could not be obtained by present methods from solid nourishing media. At one time I was inclined to regard the orange cultivation as identical with the true vaccine material, but I have subsequently found that I had inadvertently compared it with opaque lymph. Orange micrococci are two to three times larger than the spores in the true vaccine material. There is, apparently, no connection, at first sight, between the minute spore in clear lymph and the large torula-cell in opaque lymph; but if we admit developmental conditions of organisms in lymph at all, we must either regard these as impurities of accidental origin, or as products of development of organisms pre-existing in the lymph. The view that they are not accidental is strengthened by the observations of Cohn, who subjected lymph to continuous observation; and by the appearances of cultivations in solid media, especially in agar agar after incubation. If the spores can grow at all to the forms shown in the table and plates, surely it is not a great stretch of imagination to suppose that in time they may develop still further. Not only so, but they develop in circumstances favourable to reproduction, as their nutrient material becomes acid, and air is excluded. In my opinion, which I am quite aware is likely to be disputed, these peculiar bodies are probably resting-stages or

Developmental conditions

Life history of vaccine contagium.

fructification forms of micrococci, which may be described as ascococci (bladder-cocci). If these conclusions are rejected, for the sake of argument, then the forms of bacteria, described as having been obtained from vaccine and variolous lymph, must be regarded as different species; and we arrive at the curious result that we have succeeded in cultivating from lymph five forms of micrococci, each of which may be included in one of Zopf's five genera of Coccaceæ. Therefore lymph contains five kinds of micro-organisms, which is manifestly absurd; and I prefer to regard the five chief forms described, as developmental conditions or growth-forms of a single species of Sphærobacteria. The view

Unity of the germ.

that vaccine lymph contains more than one specific organism, is contradicted by the comparative ease with which pure cultivation of the vaccine and variolous disease can be carried on in the human body. Also, its power of resisting the action of other germs for an indefinitely long time, after being dried, shows that it is more likely to contaminate than to become contaminated. Then Quist has been able to multiply the material in his fluid, the essential constituent of which is the glycerine, which prevents drying of the serum and consequent stoppage of the growth. The glycerine also appears to prevent luxuriant vegetation of the spores, which are unable to develop much in size, and thus retain their power of producing a local disease. But, even in Quist's fluid, the difficulty appears to be, not to grow the spores, which is easy enough, but to prevent them from de-

veloping into more powerful growth-forms. If this difficulty has already been found in the natural material, it is probable that it will be found to even a greater extent in cultivations, either in solid or fluid media. The fact that clear lymph becomes opaque and "sour," when stored in tubes, and that experience shows it is an imperfect material for vaccination, appears to me to show that it is best to preserve it in the dry state. In this way it would be independent of the action of heat in warm climates, which must act like an incubator on fluid lymph stored in tubes. It appears, from one of Dr Buchanan's reports to the Local Government Board, that it has been found best to store calf lymph in the dry state. It seems, therefore, as if serious objection might also be taken to any method of lymph cultivation and preservation in fluid media.

Life history of vaccine contagium.

Preservation of its true bacteric form.

Müller's pure glycerine method of multiplying lymph must not, however, be overlooked, and it may still be found useful as a preservative of lymph, either natural or artificial.

I have consequently preferred to look for a substitute for vaccine lymph among the ferments, and my experiments have been confined to alcoholic yeast in the form found by distillers to possess the power of exciting fermentation in the dry state. These experiments have been inconclusive; but they appear to show that the alcoholic ferment, when inoculated, has the power of producing a temporary febrile condition in monkeys, which I have described as fermentation. Fermentation

Substitute for vaccine lymph.

Life history of vaccine contagium.

of monkeys modifies the effect of vaccination and variolation, both locally and constitutionally, the effect of both being much milder than in unprotected animals. The three kinds of inoculation have been combined successively in various orders, and the result has been to show that vaccination does not "take" after successive fermentation and variolation of a monkey, but that after simple variolation successful vaccination can be produced. Vaccination and variolation do not protect a monkey entirely from the febrile condition produced by inoculation of yeast, but nearly so. How far yeast inoculation may have the power to protect from natural smallpox I am unable to say. The fact that glycerine neither destroys the life of the vaccine ferment, nor the so-called chemical or unorganised ferment of germinating wheat and barley, suggests the idea as to whether diastase, dissolved in glycerine, might not be found to be a good vaccine material having protective properties. May not diastase really prove to consist of dried spores of a true yeast ferment?[1] Just as functional diseases are gradually being

Effect of fermentation.

Essential nature of diastase.

[1] The solution of diastase quickly alters, losing its power of converting starch. The same decomposition takes place, though slowly, in dry diastase. On boiling it with water, the decomposition is instantaneous. The action of diastase on starch is completely prevented by phosphoric acid. Essential oils, creasote, alcohol, and ether excite no retarding influence. According to Bouchardat the conversion of starch into glucose may also be effected by contact with putrid flesh, beer yeast, gastric juice, and animal membranes, which seems to imply that diastase is not a peculiar principle.—*Watt's Dict.*, 1866.

discovered to be organic, it appears possible that ferments hitherto regarded as chemical or unorganised, may be found to be really organised or physiological. Life history of vaccine contagium.

The manner in which the variolous contagium enters the body exerts a marked influence upon the severity of the disease, as is shown by the difference between natural and inoculated smallpox. The difference is apparently due to difference in the environment of the organisms. When the spores of the variolous contagium are inhaled, they find abundance of heat and moisture favourable to their development; the air-passages, in fact, may be compared to an incubator. Multiplication and absorption take place rapidly under such circumstances, and are continued in the blood till foci are formed in the capillaries of the cutis. Rindfleisch shows that there is a peculiar arrangement of the capillaries in the cutis, similar to that of the glomeruli of the kidney, where the current of blood is slower, and where consequently the organisms may obtain a firmer nidus than elsewhere. Here a focus of growth is established, which passes through the stages of papule and vesicle, when we find the organisms in the spore stage in the lymph. The vesicle may dry up at this stage; and the disease subsides at the end of the fever of invasion, without the production of secondary fever. Should the vesicles, however, become pustules, secondary fever sets in. Marson explains that probably this is not due to pyæmia from the absorption of pus; but to the absorption of some fluid Its mode of action.

Life history of vaccine contagium.

forming part of the eruption, more readily taken into the circulation than pus; or to the absorption of the products of decomposition; or to some chemical change in the contents of the pustule. I believe that, in the first instance, true pus formation does not take place in the vesicle. The opacity of the contents of the vesicle is not due at this stage to pus formation, but to development of the spores in the clear smallpox lymph into more mature organisms, such as have been demonstrated by Cohn. If this material is not discharged or dried up, spores produced by it are re-absorbed into the blood, and commence a new cycle of existence.

Its mode of action.

This explains the occurrence of many of the secondary diseases of smallpox, such as erysipelas, pneumonia, and ophthalmia, &c. Secondary eruptions after variolation and vaccination, and especially after vaccination with opaque lymph, and lymph direct from the calf, are best explained in this way, and indicate the necessity of care in the after-treatment of vaccine vesicles, so as to favour the formation of the scabs, and prevent their premature removal. Unless the vesicles dry up, on or before the eleventh day, the secondary fever in vaccination is more severe; and there is a risk of secondary eruption, entirely independent of the quality of the vaccine material employed. The practice of using vaccination-shields is to be avoided, for I invariably find that vesicles protected by a shield are surrounded by areola on the eighth day, and that the lymph is opaque. It is thus

Explanation of sequelæ.

evident that the danger of secondary symptoms may be thereby increased, in addition to the danger of erysipelas, not unfrequently incurred from their use. Ten per cent. salicylic wool, laid on lightly till the scabs are dry, and not allowed to stick in, is the best after-dressing. Life history of vaccine contagium.

The peculiar bodies resembling oil-drops, described by Cohn, have been found in calf lymph by Pfeifer, who regards them as a "Saccharomyces vaccinæ" of accidental origin. He has not found them in human lymph, but, as I have stated, they frequently occur in it; and I am inclined to think that they represent a resting stage of the vaccine organism in stored lymph, being in reality probably "Ascococci," or bladder-cocci, full of SPORES. They bear such a resemblance to yeast cells that the probability is that they are a species of acclimatised yeast. It is commonly supposed that yeast can only grow in saccharine solutions, but that is a mistake; and I state, on the authority of Professor Tyndall,[1] that very little is known as to the origin of yeast. We have seen that the yeasts are at least closely allied to the bacteria, and it is not at all improbable that a true yeast may, under certain conditions, simulate micrococci, and *vice versâ*. Saccharomyces vaccinæ.

Hallier found in variola numberless micrococci, which are said by cultivation to have germinated and passed into a known fungus, *Eurotium herbariorum*. This is a genus of Mucorini (*Hyphomycetous Fungi*), on the Eurotium.

[1] *Floating Matter of the Air.*

Life history of vaccine contagium.

distinct nature of which great doubt is thrown by recent observations of De Bary.[1]

Eurotium of authors is a mildew common upon preserved fruits, forming a whitish or yellow crust, composed of interwoven mycelium filaments, upon which are produced globular conceptacles enclosing little sacs, or asci containing several minute sporidia or spores. According to De Bary, these conceptacles are produced upon the mycelium of the Aspergillus under certain unknown conditions, and he regards them as a fruit of

Eurotium.

of the fungus different from the ordinary fructification. He states that he not only found them growing upon the continuations of the same mycelium filament, but that he has raised Aspergillus which fruited from the spores both of Aspergillus fruit and the sporidia or spores of Eurotium. He was unable to obtain Eurotium from Aspergillus spores. The dimensions of Eurotium seem to vary with the external conditions. The above curious phenomena deserve more investigation.

[1] *Micrographic Dictionary.*

CHAPTER XIV.

CHEMICAL VIEWS REGARDING FERMENTS.

Chemical views regarding ferments. Contradictory opinions.

When we turn to chemical works for information about yeast, it appears that very contradictory opinions prevail among chemists regarding it. The points which are of interest to bacteriologists are its vegetative and reproductive stages; the circumstances which modify its fermentative power; its relation to unorganised ferments; and its pure cultivation. The following abstract of opinions is taken from Watt's *Dictionary of Chemistry*:—

Cagniard de Latour. Blondeau, Mitscherlich. Pasteur.

Yeast consists of very small, round or egg-shaped balls (Cagniard de Latour); of 1μ in diameter (Blondeau). These balls are vegetable cells with elastic walls, filled with a liquid and a soft horny mass, which latter is at first attached to the walls but extends to the middle as the cell grows (Cagniard de Latour). Young cells are transparent, and almost destitute of granular contents (Mitscherlich, Pasteur). These cells multiply by gemmation (Cagniard de Latour, Mitscherlich); the newly-formed cells do not separate from the central

Chemical views regarding ferments.

cell till they have attained nearly the same size, (Pasteur). They always remain isolated, and never form ramifications or elongated cells like those of the lactous ferment (Blondeau). According to Cagniard de Latour, Turpin, and Mitscherlich, yeast-cells also increase by bursting and diffusing their granular contents through the liquid, the granules then developing into cells. Schlossberger and Pasteur did not observe this mode of formation, which is said to be inconsistent with the *uniform size* of the free yeast cells (*cf.* Plate XIII.).

Pasteur. Blondeau. Cagniard de Latour. Turpin. Mitscherlich. Schlossberger. Lüdersdorff. Wagner. C. Schmidt.

Yeast loses a considerable portion of its fermenting power by pressure, and still more by washing with water. After thorough drying, its power of exciting fermentation is for the most part destroyed (Pasteur); this statement is opposed to that of Cagniard de Latour, and the experience of the Highland smugglers. It likewise becomes inactive when heated either alone or with water.

Dried yeast excites fermentation even after cooling by solid carbonic acid (Cagniard de Latour). Yeast crushed on the grindstone no longer excites fermentation (Lüdersdorff); or only after a considerable time (Wagner); it then excites lactous fermentation (C. Schmidt). Putrefaction, poisons of fungi, alcohol, too great concentration of solutions, strong mineral acids destroy its power of exciting fermentation. Phosphoric acid alone favours it.

Two kinds.

"Ferments are of two kinds, chemical or unorganised, such as *diastase*, emulsin, &c.; and physiological or

organised, such as yeast, mycoderms, microzymes, bacteria, &c. A mode of distinguishing between the two is afforded by the action of chloroform, which kills the latter but does not produce any alteration in the former. Thus chloroform arrests the fermentation of sugar, but does not interfere with the action of emulsin or amygdalin." Chemical views regarding ferments.

"Unorganised ferments may be extracted from the vegetable and animal organs, in which they occur, by means of glycerine; in this manner diastase may be extracted from germinating wheat and barley. The ferment may be precipitated from the glycerine solution by alcohol, and obtained by repeated solution and precipitation in the form of a powder, almost entirely free from albuminoids, and very effective in converting starch into sugar" (Watt's Third Supplement, p. 779). Organised and unorganised.

Preparation of Pure Yeast free from Bacteria.—When ordinary beer-yeast is added to a filtered decoction of yeast, to which sugar-candy and alcohol have been added, the products of the action thereby set up vary with the proportions of the ingredients, more especially with the proportion of alcohol. A decoction of 40 grams of yeast in 200 c.c. of water, made up to 1 litre of water, holding in solution 100 grams of sugar-candy (Pasteur's liquid), undergoes alcoholic fermentation almost completely, on addition of a small quantity of yeast. But if the proportion of sugar be reduced to one-half, the formation of yeast-cells goes on with difficulty, while bacteria develop rapidly, and in a few

Chemical views regarding ferments.

days the liquid becomes putrid. The development of bacteria and all other disease ferments, as well as of myeoderma vini, is, however, considerably retarded by a small quantity of alcohol (2·8 per cent.), and entirely prevented by a larger quantity (5·6 per cent.). The development of yeast is also retarded by alcohol, but still goes on in solutions containing 8·2 per cent. Pure yeast may, therefore, be developed in appropriate solu-

Pure yeast.

tions containing 8·2 per cent. alcohol. With this proportion of alcohol, however, the temperature must not exceed 15° : at about 25°, even 10·6 per cent. is not sufficient to prevent completely the formation of bacteria ; but yeast grown at lower temperatures can afterwards increase, without any contamination from bacteria, in nutritive substances *free from alcohol*, even at 35°. The propagation of yeast in a solution rich in albumen, at about 30°, affords, therefore, the best criterion of its perfect freedom from bacteria, a point not easy to determine by microscopic examination (Traube, *Deut. Chem. Ges. Ber.*, ix. 183, 1239 ; Watt's Third Supplement, p. 783).

Its growth in albuminous solutions.

It appears, then, that Hallier had also observed the globular bodies in lymph, which he compared to Eurotium of fruits, and chemical researches place it beyond question that yeast can grow and multiply in richly albuminous solutions, so that, under certain favourable circumstances, it is quite possible that it may vegetate in the animal body. In text-books it is usual to find the statement that yeast grows by gemmation, and

there is no doubt that it does do so. In my opinion, this, however, only represents the stage of vegetation, in which there is no true reproduction, and this view is taken by Cagniard de Latour, who thinks that multiplication also takes place by bursting of the cells and scattering of their contents in the fluid, in fact by spore-production. Then, although young cells are said to be homogeneous, yet it cannot be said that dried yeast cells present this appearance (see Plate XIII.), since they evidently contain what must be regarded as reproductive elements in their interior. In various ways, it has been distinctly proved that drying does not deprive yeast of its vitality, so that it is probable that spore-production takes place during the process. It would be interesting to know from chemists whether the acidity of opaque vaccine lymph is due to the presence of phosphoric acid, which is said to favour its development. If, therefore, Cagniard de Latour's view as to the reproduction of yeast by spore-formation be correct, we must admit that such spores, being much more minute than the vegetative yeast cells, may exist, much more universally than is at present admitted, in a form which cannot be distinguished by size or arrangement from the Sphærobacteria. Koch has found that no other bacterial forms give the same colour reaction as the tubercle bacilli; but *spores* may, if fresh. Dr Gaffky discovered that the spores of moulds became deep blue, and a certain kind of yeast also seems to stain in the same way. This observation is of extreme

Chemical views regarding ferments.

Pure yeast.

Its vitality and mode of reproduction.

Its staining reaction.

Chemical views regarding ferments.

interest, because the best stain for both fresh clear lymph and opaque vaccine and variolous lymph is the aniline methyl-violet stain used for tubercle bacilli, and the demonstration is equally difficult. The method

Diastase.

employed by chemists to extract diastase from germinating wheat and barley does not appear to prove completely the inorganic nature of the product. The ferment is dissolved by glycerine and then precipitated by alcohol, and this process is repeated several times. But we have seen that the vaccine ferment is not destroyed by glycerine, and that the alcoholic ferment is not entirely destroyed by alcohol, so that neither glycerine nor

Probably a vital ferment.

alcohol have the power of destroying some organised ferments, but only of inhibiting their multiplication; and it appears that diastase retains its power of converting starch into sugar after being thus treated. It resembles apparently dry powdery yeast, which, notwithstanding, retains its power of exciting fermentation. Then the power which malt possesses of transforming ten times its weight or more of the starch in fresh barley into sugar and dextrine, points to an organised rather than a chemical ferment as the probable cause of the transformation. The two materials require to be constantly stirred at a high temperature till the transformation is complete. It appears probable that during this time there is an actual increase in the quantity of ferment, which cannot well be accounted for on the chemical hypothesis, but which is much more easily explained by the supposition that

the diastase ferment may consist of spores, and be organised; "the balance of evidence at present being in favour of the view which regards the action of living organisms as essential to the commencement of the fermentative process." Chemists, twenty years ago, admitted that they knew nothing whatever of the mode of action of these organisms.

Chemical views regarding ferments.

CHAPTER XV.

SUMMARY OF RESULTS.

General Summary.

1. THE bacteric form of the true vaccine contagium is to be found in the minute isolated spores of micrococci, which are suspended in standard vaccine lymph, and this form must be preserved in artificial vaccine materials.

2. Opaque vaccine lymph contains more mature bacteric forms developed from the spores in clear lymph, and is to be regarded as a natural cultivation of the true vaccine contagium.

3. The torulæ described in opaque lymph do not exist in clear lymph, and their significance is doubtful, but most probably they represent a resting or reproductive stage of existence of the vaccine contagium.

4. Stored lymph undergoes acid fermentation, unless preserved in a dry state, the alkalinity of standard lymph being favourable to the continued multiplication of the spores which it contains.

5. The chemical change thus produced in lymph suggests the opinion that the vaccine contagium is a

vegetable ferment which has become acclimatised in warm-blooded animals. General Summary.

6. The bacteric forms found in standard and opaque lymph have undoubted pathogenic properties.

7. The vaccine contagium can be isolated and cultivated apart from the animal body, in both solid and fluid media.

8. By artificial cultivation in solid media, the bacteric forms found in opaque lymph are reproduced, but in much greater quantity. These forms do not exist in standard vaccine lymph.

9. By artificial cultivation in Quist's fluid, the bacteric forms found in standard lymph appear to be reproduced, the further development of these into growth-forms being probably prevented by the action of the glycerine which it contains.

10. The corresponding variolous materials also contain the embryonic and mature bacteric forms found in standard vaccine lymph and cultivations.

11. The properties of pure dried yeast and diastase are very similar to those of the vaccine contagium.

12. Experimental vaccination proves that natural and artificial vaccine materials differ in potency as well as in composition.

13. The embryonic natural material produces perfect local results, seldom secondary lesions.

14. The mature natural material produces more violent and imperfect local results, which are not unfrequently followed by secondary lesions.

General Summary.

15. The developmental bacteric forms of the vaccine contagium, obtained by artificial cultivation, produce a rapid constitutional action, followed by secondary lesions, without any local result at the points of inoculation.

16. Artificial cultivations of variola have a similar action, and the contagium apparently becomes less virulent.

17. The pathogenic properties of the vaccine and variolous materials depend on the embryonic and mature forms of bacteria which they contain.

18. The recognition of these pathogenic properties becomes difficult when only a constitutional result is produced by the modified materials.

19. Experimental inoculation of dried yeast produces local irritation and fever, which shows that ordinary ferments have some pathogenic property. It appears to modify the action of the vaccine and variolous contagia, but is not fully protective against the action of either.

20. It is probable that both yeast and diastase can become acclimatised in animals, and retain their power of exciting fermentation.

21. Vaccination appears to consist essentially in the artificial growth of a vegetable ferment in an animal, under circumstances unfavourable to its rapid multiplication.

22. Immunity is produced by gradual fermentation of the blood, and requires for its full explanation the combination of the Exhaustion, Ptomaïne and Meta-

bolism Theories of protection. Metabolism of the blood-cells must take place during the fermentative process. These transformed cells then become vital ferments capable of effecting a similar metabolism of new material. This power gradually becomes exhausted, and the immunity ceases. General Summary.

23. Attenuation of the virus appears to be best explained by spore-production, as the true vaccine contagium has been demonstrated to consist of spores. The difficulty in obtaining reliable vaccine material consists in preventing these from developing into more mature bacteric forms, and this difficulty has to be overcome in artificial cultivations.

24. Standard vaccine lymph, carefully propagated as at present, is therefore, in my opinion, the best and most convenient material that can be obtained for vaccination. My observations appear to show that when it is taken from a typical Jennerian vesicle, it is a material "in the contagion of which no second principle can possibly reside."

APPENDIX A.

PRELIMINARY PAPER.

READ BEFORE THE ROYAL SOCIETY OF EDINBURGH, FEBRUARY 15, 1886.

(Communicated by Professor CHIENE.)

THE LIFE HISTORY OF THE MICRO-ORGANISMS ASSOCIATED WITH VARIOLA AND VACCINIA: An Abstract of Results obtained from a Study of Small-Pox and Vaccination in the Surgical Laboratory of the University of Edinburgh. By J. B. BUIST, M.D., F.R.C.P. Edinburgh.

ORIGIN OF INQUIRY.

THE following investigations were undertaken with the view of ascertaining the nature and cause of opacity in vaccine lymph. According to the authorities on the subject, clear fresh lymph is a perfect material for vaccination, while opaque lymph, fresh or stored, is an imperfect material, but hitherto the nature of the difference between them has not been explained satisfactorily. It has been maintained by many that

opaque lymph is a satisfactory material for the purpose of vaccination, but this view is not endorsed by the National Vaccine Establishment. In April, last year, Professor Chiene suggested to me that the cause of opacity in lymph was to be found in a "germ," and he cordially assented to my request for assistance in making the necessary cultivations in order to determine its nature. The cultivations detailed were made by his assistant, Mr Hare, whose intimate acquaintance with modern methods of bacteriological research has saved much time and labour. The responsibility of the observations recorded rests upon me entirely.

A preliminary microscopic examination of clear and opaque vaccine lymph led to no definite result, as the appearances observed could not be explained. It was therefore decided to examine—

I. Empty Commercial Vaccine Tubes for "germs."
II. Cultivations of Vaccine Lymph.
    a, By the naked-eye; *b*, by the microscope.
III. Cultivations of Variolous Lymph.
    a, By the naked-eye; *b*, by the microscope.
IV. Clear and Opaque Vaccine and Variolous Lymph.
V. The Results of Experimental Vaccination.

I. Examination of Empty Commercial Vaccine Tubes.

The problem to be solved was whether such tubes

contain germinal matter. To determine this, two series of experiments were undertaken.

1. Sealed commercial tubes were introduced with aseptic precautions into beakers containing sterile nutrient fluid. Continued sterility of the fluid was proved by subsequent incubation at 35° C. The tubes were then broken with aseptic precautions. Subsequent incubation showed that more than half of the beakers containing broken tubes became cloudy.

Where the sealed tubes were left unbroken, but where the fluid had merely been stirred with a sterile glass rod, one out of six only became cloudy.

2. Commercial and sterilised vaccine tubes were charged with sterile and non-sterile fluid, with and without spray, in imitation of the conditions under which vaccine lymph might be stored. The result showed that sterility of the tube and of the fluid and the use of the spray were necessary to prevent the occurrence of opacity. Where these conditions were absent opacity occurred in every tube.

The fact that a large proportion of the beakers containing broken tubes showed no change in the fluid, led to the conclusion that the amount of germinal matter in commercial tubes was very small. Besides, the amount of opacity in the fluid in the second series of experiments was very much less than that observed in vaccine lymph. It was therefore concluded that the contents of the tubes had very little to do with the production of opacity in vaccine lymph. This con-

clusion is concurred in by Mr Farn, of the National Vaccine Establishment.

II. Examination of Cultivations of Vaccine Lymph.

All the cultivations were made by Mr Hare with aseptic precautions. The material was transferred directly from the vesicles to the cultivating medium. All the successful cultivations were got from clear lymph. None were got from opaque lymph. The appearances were noted in each cultivation on three separate occasions.

An analysis of the annexed table shows that in primary cultivations the colour of the growth, with scarcely any exception, was white on the fourth day. Primary cultivations in Koch's jelly were invariably white, in the form of "cocoons." On the eighth day yellow and orange colour appeared in a certain proportion, but the prevailing colour was still white. After this the principal colours were white and yellow, only one cultivation being of an orange colour. We had thus three distinct growths, white, yellow, and orange, as described by other observers. Secondary cultivations were made from certain of the tubes which had been opened for microscopic examination. In only one case out of six were the same colours reproduced. Change of colour in the growth was thus observed to take place both in primary and secondary cultivations. It is difficult to explain how this can take place on

TABLE *showing Macroscopical Appearances of Vaccine Cultivations.*

No.	Name of Tube.	4th Day.	8th Day.	Result.
1	I.A, agar.	White.	Faint yellow.	Dull yellow.
2	I.B, ,,	,,	,,	,,
3	II.A, ,,	,,	Yellowish-green.	Bright yellow.
4	II.B, ,,	,,	,,	,,
5	I. ,,	No report.	No report.	Dull orange.
6	II. ,,	,,	,,	White and yellow.
7	I. jelly.	,,	Or., white, yel.	Orange.
8	I.B, ,,	,,	,,	,,
9	II.A, ,,	,,	White and yel.	Yellow.
10	II.B, ,,	,,	,,	,,
11	II. ,,	,,	No report.	,,
12	I.A′, agar.	Secondary.	,,	Orange.
13	II.B′, ,,	,,	,,	Yellow and white.
14	I.B′, jelly.	,,	,,	Bright orange.
15	II.A′, ,,	,,	,,	Yellow and white.
16	I. jelly, yellow.	,,	Orange.	Orange.
17	I. jelly, white.	,,	,,	,,
18	III.B, agar.	White.	Dull orange.	Dull orange.
19	III.A, ,,	,,	White.	White.
20	III.A, jelly.	No reaction.	No reaction.	No reaction.
21	III.B, ,,	,,	,,	,,
22	IV.A, agar.	From opaque lymph.		,,
23	IV.B, ,,	,,	,,	,,
24	IV.A, jelly.	,,	,,	,,
25	IV.B, ,,	,,	,,	,,
26	V.A, agar.	White.	White.	White.
27	V.B, ,,	,,	,,	,,
28	V.A, jelly.	No report.	No report.	White and yellow.
29	V.B, ,,	,,	,,	,,
30	VI.A, agar.	Greyish-blue.	Greyish-white.	Thick wh. growth.
31	VI.B, ,,	,,	,,	,,
32	VI.A, jelly.	White.	White.	Jelly liquefied.
33	VI.B, ,,	,,	,,	Yellow.
34	VII.A, jelly.	,,	,,	White.
35	VII.B, agar	White.	Dull orange.	Dull or. & brown.
36	VIII.A, agar.	Wh. & dull or.	White.	Strong wh. growth.
37	VIII.B, agar.	,,	Dull orange.	Dull orange.
38	IX.A, jelly.	White.	White and yel.	White and yellow.
39	IX.B, agar.	,,	White.	Yellow.
40	X.A, agar.	,,	,,	White and yellow.
41	X.B, ,,	,,	White & dull or.	,,
42	XI. agar.	,,	White.	Wh.,yel.,& brown.

the supposition that we have three different organisms to deal with.

2. *Histological Examination of Vaccine Cultivations.*

A. Orange cultivation showed swarms of minute spherical "micrococci" without definite arrangement.

B. White cultivations showed single, double, and triple micrococci, and in one case a sarcina form. The micrococci were larger in cultivations which had been incubated.

C. Yellow cultivations showed dumb-bell and sarcina-form micrococci. Here also the incubated cultivations showed larger torula-form micrococci.

We could not explain these different appearances. It was supposed that we had three different organisms, any one of which might be the immediate cause of vaccinia. Other preparations of vaccine lymph, clear and opaque, were now made.

In clear lymph certain minute badly stained spherical bodies were recognised, similar to those of the orange vaccine cultivation, but I am indebted to Dr Francis Troup for the first clear demonstration of them in vaccine lymph. I then saw that the "micrococci" in clear lymph and orange "vaccine" were identical. Opaque lymph, after being kept some time, showed large spherical transparent bodies like oil drops, but their nature was a mystery. Even after examining vaccine cultivations, we were as far off as ever from being able to explain the nature and cause of opacity in

lymph. I therefore suggested that we should cultivate variola.

III. Examination of Cultivations of Variolous Lymph.

As there were no cases of smallpox in Edinburgh suitable for our purpose, it became necessary to visit the Hospital Ships at Purfleet, on the Thames, to obtain variolous cultivations. I have to acknowledge the readiness with which Dr Birdwood placed the material at his disposal at our service, and also the able assistance given by Messrs Bott and Clatworthy, the resident physicians.

The variolous cultivations may be conveniently divided into three classes:—

1. Where no reaction or growth occurred.
2. Where growth occurred without liquefaction.
3. Where growth was accompanied by liquefaction.

The first class is of value as showing the care with which contamination of the media was prevented. The third class was excluded from present comparison with vaccine cultivations, by the occurrence of liquefaction of the media. The second class may fairly be compared with vaccine cultivations. But we had found that the most definite and easily recognised form of growth was the "cocoon" in Koch's jelly. Our series is therefore reduced to cultivations of variola, showing this distinct mode of growth. Nos. 11, 12, and 39 only showed a large definite "cocoon" growth without liquefaction.

These were selected as parallel, and probably identical, with vaccine cultivations of the same appearance. The questions to be decided were, as to their histological appearances, physiological action, and contagiousness.

2. *Histological Investigation of Variolous Cultivations.*

A. White "variola" showed single, double, and triple micrococci, identical with white "vaccine."

B. Clear variolous lymph showed minute spherical bodies, similar if not identical with those of clear vaccine lymph and orange "vaccine."

C. Opaque variolous lymph showed large torula-looking "micrococci," as well as smaller forms.

IV. Examination of Clear and Opaque Vaccine and Variolous Lymph.

On comparing clear and opaque variolous lymph with clear and opaque vaccine lymph, the micro-organisms in each appeared identical. My observations are thus in accordance with the description given by Cohn, quoted by Burdon Sanderson in his Report on the Intimate Pathology of Contagion. Cohn was unable to say whether there was any connection between the bodies in clear and opaque lymph; but Zopf, in his work *Die Spaltpilze*, 1885, states that the opacity "consists in cell-rows and masses resulting from the continuous division of the cocci." He recognises no other modes of development.

TABLE *showing the Macroscopical Appearances of Cultivations of Variolous Lymph in Solid Media.*

Source.	No.	Name of Tube.	8th day.	13th day.	Result.
Early pustular or pustular.	1	Aa, jelly.	No reaction.	No reaction.	No reaction.
	2	Ab, ,,	,,	,,	,,
	3	Ac, ,,	,,	,,	,,
	4	Ad, ,,	,,	,,	,,
	5	Ae, agar.	,,	,,	,,
	6	Af, ,,	,,	,,	,,
	7	Ag, ,,	,,	,,	White.
	8	Ah, ,,	,,	,,	,,
Blood, papulo-vesicular.	9	Ai, serum	White riband.	White.	,,
	10	Aj, ,,	No reaction.	Trace of white.	,,
	11	Ba, jelly.	White cocoon.	White cocoon.	Pure white.
	12	Bb, ,,	,,	,,	,,
	13	Bc, ,,	No reaction.	No reaction.	No reaction.
	14	Bd, Agar.	White cloud.	White cloud.	White cloud.
	15	Be, ,,	,,	,,	,,
	16	Bf, ,,	No reaction.	No reaction.	No reaction.
	17	Bg, serum	,,	,,	,,
	18	Bh, ,,	,,	,,	,,
Papulo-vesicular. Vesicular.	19	Ca, agar.	White zone.	White zone.	White zone.
	20	Cb, ,,	White cloud.	White cloud.	White cloud.
	21	Cc, ,,	White zone.	White zone.	White zone.
	22	Cd, serum	White cloud.	White cloud.	White cloud.
	23	Ce, ,,	,,	,,	,,
	24	Da, jelly.	White cocoons.	White.	White.
	25	Db, ,,	Liquef	action, and white P	recipitate.
	26	Dc, ,,	No reaction.	No reaction.	No reaction.
	27	Dd, agar.	White.	White.	Faint white.
	28	De, ,,	No reaction.	,,	White.
	29	Df, ,,	,,	No reaction.	No reaction.
	30	Dg, serum	,,	,,	,,
	31	Dh, ,,	White riband.	White.	White.

Cultivations of Variolous Lymph—continued.

Source.	No	Name of Tube.	8th Day.	13th Day.	Result.
Vesicular.	32	Ea, jelly.	No reaction.	No reaction.	No reaction.
	33	Eb, ,,	White cocoon.	White cocoon.	White.
	34	Ec, ,,	,,	,,	Liquefying.
	35	Ed, ,,	,,	White, liquefying	with white p.p.
	36	Ee, ,,	,,	White.	White.
	37	Ef, ,,	Cocoons and	liquefying globe,	with white p.p.
	38	Eg, ,,	,,	,,	,,
	39	Eh, ,,	White cocoon.	Larger white.	Pure white.
	40	Ei, ,,	No reaction.	Small cocoon.	White.
	41	Ej, ,,	White cocoon.	Globe of liquefac.,	with orange p.p.
	42	Ek, ,,	,,	White.	Faint white.
	43	El, ,,	Cocoons.	Globe of liquefac.,	with yel. p.p.
	44	Em, agar.	White cloud.	White cloud.	White cloud.
	45	En, ,,	White zone.	Thick white zone.	White.
	46	Eo, ,,	Liquefying.	Liquefying.	Liquefying.
	47	Ep, ,,	White cloud.	White.	White.
	48	Eq, ,,	,,	,,	,,
	49	Er, ,,	Liquefying ?	Liquefying ?	Solid white.
	50	Es, ,,	White cloud.	White cloud.	Wh. with or.
	51	Et, ,,	No reaction.	No reaction.	No reaction.
	52	Eu, serum	White.	White.	White riband.
	53	Ev, ,,	No reaction.	No reaction.	No reaction.
Papulo-vesicular.	54	Fa, jelly.	Faint white.	White.	Faint white.
	55	Fb, ,,	Wh. cocoons.	,,	,,
	56	Fc, ,,	,,	,,	Wh. cocoons.
	57	Fd, ,,	No reaction.	No reaction.	No reaction.
	58	Fe, ,,	White.	White.	Liquefac. and white p.p.
	59	Ff, ,,	,,	,,	Faint growth.
	60	Fg, agar.	,,	Thick white.	White cloud.
	61	Fh, ,,	,,	,,	,,
	62	Fi, ,,	,,	,,	,,
	63	Fj, ,,	No reaction.	No reaction.	No reaction.
	64	Fk, ,,	,,	,,	,,
	65	Fl, scrum.	White riband.	White riband.	White riband.
	66	Fm, ,,	,,	,,	,,
Blue.	67	Ga, jelly.	Funnel-shaped	liquefaction, with	white p.p.
	68	Gb, ,,	,,	,,	,,
	69	Gc, ,,	,,	,,	,,
	70	Gd, ,,	,,	,,	,,
	71	Ge, agar.	Or. or ochre.	Cocoons solid.	Ochre cocoons.
	72	Gf, ,,	,,	,,	,,
	73	Gg, ,,	,,	,,	,,
	74	Gh, ,,	White cloud.	White cloud.	White cloud.
	75	Gi, ,,	White and or.	White and ochre.	Wh. and or.

V. Experimental Vaccination.

We divide the experiments into four series—

I. Vaccination of Calves.
II. Vaccination of Guinea-pigs.
III. Vaccination of Monkeys.
IV. Contagion Experiment.

Ten experiments were performed, as is shown by the following table:—

No.	Vaccine Material.	Vaccinifer.	Result.
I.	White, yellow, and orange vaccine.	Calf.	Pustular eruption on head.
II.	Opaque variolous lymph.	Calf.	Pustular eruption on back.
III.	Vesicular variolous lymph.	Calf I.	Protected by vaccine I.
IV.	Pustular variolous lymph.	Calf II.	Protected by variolation II.
V.	Orange vaccine.	Guinea-pig.	Local scab.
VI.	White vaccine.	Guinea-pig.	No local result. Constitutional?
VII.	Yellow vaccine.	Guinea-pig.	No local result. Constitutional?
VIII.	Clear vaccine lymph.	Monkey.	Four typical vaccine vesicles.
IX.	White variolous cocoon.	Monkey.	Eight pocks. No local result.
X.	Modified variola IX.	Monkey.	Not contagious.

An analysis of this table shows that orange "vaccine" produces a local scab in a guinea-pig, and that clear vaccine lymph produces typical vaccination in monkeys. Vaccine cultivations and opaque variolous lymph produce an eruptive fever in calves when inoculated. No local result is produced. White variolous cultivation produces a mild variolous eruption in a monkey, which

does not appear to be contagious to another monkey. No local result is produced at the points of inoculation. Protection is afforded from the poison of variola by previous variolation and vaccination with vaccine cultivations. White and yellow "vaccine" produce no local result in guinea-pigs. They probably produce a mild constitutional result. The experiments appear to show that different vaccine materials possess different degrees of potency.

Classification of Micro-Organisms.

In attempting to trace the life history of the vaccine and variolous organisms from the preceding observations we are confronted by a preliminary difficulty. At present the fission-fungi are undergoing the most minute investigation, and the result has been attempts to improve upon the classifications of Cohn and Naegeli. Naegeli held that the bacteria were allied to yeasts, and should therefore be included in the class of fungi. The difficulty in accepting this view arose from the fact that fungi were supposed to be destitute of colouring matter. Owing to this, Cohn placed them among algæ, but the tendency now is to amalgamate the colourless fungi (bacteria) and the colour-producing algæ (bacteria) into one group, the Thallophytes (Sachs). The latest classifications of Flugge, 1883, and Zopf, 1885, appear to me to be unsatisfactory, and I prefer to follow the classification of Cohn into four tribes, including six genera. He believes that the form or

shape characteristic of each tribe is adhered to throughout the life of the organism. Thus, a micrococcus cannot be transformed into a bacterium or bacillus, but retains its spherical shape. We have only to do at present with Cohn's first group, the Sphærobacteria, comprising one genus, the micrococci. While, however, Cohn has settled the main lines upon which the classification of Schizomycetes should be based, he admits that his classification of the genera into species is defective. This is due to deficient knowledge of their physiological action and modes of reproduction. Thus, micrococci having the same appearance may have different effects. Cohn divides his genus, Micrococcus, into three physiological species—(1) Chromogenic, (2) Zymogenic, (3) Pathogenic. He places the Micrococcus Vaccinæ among the pathogenic micrococci, but the record of the cultivations just detailed shows that the vaccine organism is also chromogenic, so that it might equally well be classed among these coloured micrococci. At the same time, he admits that differences in arrangement and size are unreliable data upon which to found the classification of species. A necessity, therefore, arises for the acceptance to some extent of the theory of pleomorphism (Tulasne), *i.e.*, that the same plant can occur under two or more forms, as well with respect to the organs of vegetation as to those of reproduction. Applying this theory to the bacteria, we find that a single species may show various forms in the course of its life-cycle. This has been shown by the researches

of De Bary, Zopf, Dallinger, Douglas Cunningham, Ewart and Geddes, and others.

The propagation of fungi takes place asexually in three ways—1. By free cell formation (asci, thecæ, spore-pouches). 2. Constriction (basidians). By cell-fission or gemmation. Spores are the chief means of the spread of fungi (Wagner).

Surrounding media modify the form and mode of fructification of fungi, some increasing and others diminishing spore-formation, or we may have the fungus dividing into yeast forms. Schwann, Pasteur, and others consider the yeast fungi as organisms *sui generis*, arising in fermentable liquids from their own specific germs.

Hallier, Hoffman, and others consider that they are only conditions, especially of mould fungi occurring in fermentable liquids, particularly the spore forms, which fructify in the atmosphere in other forms. They originate likewise from spores or from yeast cells themselves when they reach a liquid. Hoffman thinks that the genera of Schizomycetes, described by Ehrenberg, Pasteur, &c., pass into one another, peculiarities which are to be held as characteristic of the species, and which change in the course of development according to the change in the external conditions of life (Wagner). As we have seen, Cohn maintains that such genera are distinct throughout life. Species may show various sizes and different pigments besides differing in their effects. Zopf is a warm

supporter of the theory of pleomorphism. Crookshank (*Bacteriology*, 1886), who adopts Zopf's classification, says that in classifying species of bacteria we must take into account—1. Macroscopical appearances in various nutrient media. 2. Character of their colonies under a low power, in plate cultivations. 3. Microscopical appearances of the organisms themselves. 4. Their physiological action. These desiderata, however, scarcely satisfy the requirements of the case, and I beg leave to add—5. The study of their modes of development and reproduction in each species.

In this way we may be able to make out the life history of an organism. Recent observations by Lister, Neelsen, Zopf, Van Tieghem, Klein, and Hauser, are said (Crookshank) to show that the orders of Cohn pass into one another. It is due to Lister to say, however, that he retracted his first opinion with regard to the bacillus of black milk, and expressed a doubt as to whether he had not got a mixture of organisms. If this were true, of course, Cohn's classification of genera would fall to the ground. Cohn's strong point is that he opposes change of shape in genera. He admits differences in size and arrangement and colour as well as physiological action, in the different species of each genus. The truth appears to be, that while the various genera retain their globular, oval, rod-shaped, or spiral form throughout life, the different species described as cocci, rods, threads, and spirals may be merely stages of growth of a single organism. At the same time

these various forms may produce different physiological effects. We find so-called "cocci" described as growing into torulæ or rods. But we find that rods are spore-bearing, so that it appears rather a misnomer to call such spores cocci. Should they not be called spores of bacilli? It is evident that a gap has to be filled up between the higher torula-form and the spore. The researches of Dr Douglas Cunningham upon the micro-organisms found in the intestinal canal show that they have an active and a resting stage. In suitable media, the "zoospores" multiply till the material for their growth is exhausted, when the medium is acid, and the micro-organisms cannot develop further. When transferred to an alkaline medium they at once become active.

The mode of reproduction of a "minute septic organism" has been well described by the Rev. W. H. Dallinger, in a remarkable paper communicated to the Royal Society of London in 1878 by Professor Huxley. He finds that the bacterium grows to an ovoid body, of very delicate nature, which bursts and gradually collapses, scattering very minute spores. By continuous observation, he has seen the spore grow again into the ovoid body, which was seen to burst again as before. Mr Dallinger worked with very high powers, × 3000. Similar observations with regard to the life history of bacteria have been made by Professor Cossar Ewart, but he does not emphasise the resting stage. Mr P. Geddes points out that this occurs in many instances in the cycle of cell-life.

TABLE *showing the Histological Appearances of the Vaccine Organism in Solid and Fluid Media.*

No.	Med.	Material.	Forms.	Corresponding Forms.
1	Fluid.	Clear lymph.		Burdon-Sanderson's 'microzyme.' Cohn's spheroidal corpuscle.
2	,,	,,		Cohn's simple corpuscle or M. Vaccinæ.
3	Solid.	White in jelly.		Cohn's 'pairs of corpuscles.'
4	,,	White in agar.		Cohn's 'groups' or 'clumps.'
5	,,	Yellow in jelly.		Cohn's 'groups resembling sarcinæ.'
6	,,	Yellow in agar.		Cohn's 'larger aggregations' or 'clumps.'
7	Fluid.	Opaque lymph.		Burdon-Sanderson's 'semifluid material' or 'oil-drops.' Cohn's "refractive cells resembling oil-drops.'
8	Solid.	Orange, 8th day.		Burdon-Sanderson's 'microzyme.' Cohn's 'minute spheroidal corpuscle.'

TABLE *showing Appearances of the Variolous Organism in Solid and Fluid Media (after Cohn).*

No.	Med.	Material.	Forms.	Corresponding Forms.
1	Fluid.	Clear lymph.		Cohn's minute sphere.
2	,,	,,		Cohn's simple corpuscle.
3	Solid.	White in jelly.		Cohn's pairs of corpuscles.
4	,,	,,		Cohn's 'groups' or 'clumps.'
5	Fluid.	Opaque lymph.		Cohn's 'groups resembling sarcinæ."
6	,,	,,		Cohn's 'larger aggregations.'
7	,,	,,		Cohn's 'refractive cells resembling oil-drops.'
8				

Life History of the Vaccine Organism.

In traeing this I shall follow the method reeommended above by Crookshank, as my observations fall naturally under his headings.

1. *Macroscopical Appearances of Vaccine Cultivations.*—We found that the best medium for making observations was Koeh's jelly. Here we obtained three definite eoloured growths. The eolours on the surfaee of the jelly were white, yellow, and orange. Below the surface they were white and yellow. No orange colour appeared below the surface. The shape of the colonies was oval or like a eoeoon. Sometimes these were aggregated together, and irregular on the surface. They grow from inoeulations of elear lymph only.

2. *Character of their Colonies.*—The "colonies" have been well deseribed by Cohn, in 1872, in preserved lymph. He saw the minute spherical corpusele of fresh elear lymph sueeeeded by "simple corpuscles, double corpuseles, rows and heaps or masses of eorpuseles." He saw the eorpuseles become larger, until at last the lymph contained separate large globular homogeneous transparent bodies like oil-drops. I ean eonfirm those observations as eorreet, and I agree with Zopf (1885) in thinking that they can only proeeed from continuous division and growth of the organisms in clear lymph. Opacity of lymph is produeed in this way. I observed, however, that the large torula-looking bodies disappeared from fresh preparations within

twelve hours, and next day both stained and unstained preparations showed large masses, which had apparently grown from them by budding. I think that this appearance is produced by the bursting of the globules, but I have not seen this take place. I think that these large cells contain spores, and that they are developed from the organisms in clear lymph. If these bodies originated in accidental contamination of the lymph, it is difficult to see why they should be of constant occurrence in stored lymph, and not only so, but also in opaque lymph within the vesicles. Then they did not appear in the other nutrient fluid used to imitate vaccine lymph in the tube experiments. The stages of growth between the embryonic and the mature form have been accurately traced by Cohn, both in vaccine and variolous lymph. The larger forms are never found in fresh clear lymph.

With regard to the colonies in solid media, the first appearance is white, even when the growth is of some size. The change of colour takes place gradually, and it remains yellow. Spore-formation, as shown by the bright orange colour, is seldom seen in solid media. I am unable to explain this, except that the environment is unfavourable to the production of spores, while it is favourable to the ordinary vegetative growth. Then, when secondary cultivations were made, the same colour was only once reproduced. If change occurred, it was in the direction of more distinctness in the colour, apparently showing that the change of medium

was more favourable for spore-production. It would be interesting to know whether coloured growths of vaccine lymph are alkaline or acid in reaction. If the orange growth is composed of spores, it should correspond to the alkaline clear lymph. At the same time the white and yellow should show an acid reaction similar to that given by opaque lymph. We may suppose the yellow colour to be produced by a mixture, of vegetative and spore growth, *i.e.*, white and orange.

3. *Microscopic Appearances of the Organisms.*—My observations show that the size of the organisms increases with the age of the cultivations. Incubation has also some effect, and one medium may be more suitable than another for its growth. Thus in Koch's jelly the micrococci were smaller than those in agar after incubation. As the material was the same, this could only be explained by more rapid growth. Thus we find that the same organism, derived from the same source, presents different appearances according to the environment and other favourable circumstances. It does not follow, however, that the same forms will produce the same effects. Nor does it follow that different-sized organisms will not produce similar physiological effects. The truth seems to be that the larger organism is the more powerful. The embryonic form is milder in action.

4. *Physiological Action.*—The results of experiments, although few in number, appear to show that the embryonic form is milder in action than the more

mature form of organism. Thus clear vaccine lymph and orange vaccine, both containing minute organisms, produced only a local result. Opaque variolous lymph and white and yellow vaccine cultivations, and white variolous cultivations, containing larger organisms not found in clear lymph, produce an eruptive fever. They appear to be more powerful in action. If the organisms do not grow from the minute spheres in clear lymph, where do they come from? Are there several kinds of minute spheres in clear lymph? Even if so, according to the opposite view, they could not change their size and arrangement by cultivation only.

5. *Mode of Development and Reproduction.*—My observations appear to confirm Cohn's opinion that genera of bacteria retain their shape throughout their life history. Micrococci remain micrococci, but they may present variations in size, arrangement, and colour, and their physiological action may become more powerful. Definite production of bright colours appears to be associated with spore formation. The large transparent delicate torula found in fluid media represents the organism in its resting stage, as described by Dallinger and Douglas Cunningham. The active stage commences when this mature organism is transplanted to a suitable medium. Then spore-production immediately becomes complete, and a fresh cycle of growth commences. I cannot say where these spore-bearing cells originate, but it is possible that they come from yeasts or moulds.

Summary of Results.

The following conclusions appear to be warranted by the results of the inquiry just detailed. Bacteriologists are still at variance as to whether bacteria spring from higher forms, and grow into them again. So far as the globular or sphæro-bacteria are concerned, the only mode of reproduction at present recognised is fission occurring in one or more directions. Different morphological appearances and colours are held to be distinctive of different species of micrococci, but it appears probable that a careful study of the physiological action and mode of reproduction is required before a satisfactory classification can be obtained. Continuous observation of each organism from its embryonic to its mature form is necessary. Cultivation of the organisms, in both solid and fluid media, also appears to be imperative. It is also necessary to distinguish between local and constitutional results of experimental inoculation with cultivations.

We conclude, then,

1. That commercial vaccine tubes contain germinal matter, but in small amount, and that it assists in the production of opacity in lymph.

2. Clear vaccine lymph, clear variolous lymph, and orange "vaccine" contain probably, not micrococci, but the spores of micrococci. Inoculation with these materials produces local irritation by the growth of the "spore" into a more mature form of organism.

3. Opaque vaccine lymph and opaque variolous lymph contain larger, more refractive bodies of various sizes, which are probably developed from the spores in clear lymph. They increase in size and number in proportion to the length of time the lymph has been kept. After a few months large torulæ crowd the field in microscopic preparations. Such lymph has an acid reaction, and when inoculated it produces either violent local action, *i.e.*, accelerated vaccination, or we may have no local action, but secondary eruption in some other part of the body. This is probably due to rapid spore-production by the torulæ. The spores are probably absorbed into the blood, and rapidly grow there, until they are arrested in the capillaries, where they continue to grow and set up irritation, ending in the production of vesicles or pustules. From these, secondary foci may again be produced. This would account for the appearance of periodic secondary eruptions after vaccination. The growth from the spore to the torula explains the occurrence of opacity in lymph. Drying the lymph prevents this growth, and also deterioration of the true vaccine material.

4. White, yellow, and dull ochre or orange cultivation of vaccine lymph in solid media, appear to represent stages of growth between the spore and the torula. They correspond to the refractive corpuscles and cells described by Cohn in vaccine lymph. When inoculated, they produce in calves eruptive fever. They do not produce a local result.

5. White variolous cultivations in solid media appear to represent stages of growth between the spore in clear variolous lymph and the torula in opaque variolous material. When inoculated, a mild eruptive fever was produced in a monkey. This was not contagious to another monkey. The organisms correspond to the refractive corpuscles of Cohn. Cultivations of variola and vaccinia in solid media appear to produce mild eruptive fever. They do not produce a local result.

6. Inoculation with vaccine and variolous cultivations protects from a subsequent attack of variola in calves.

7. The best or perfect material for vaccination appears to be that which contains spores only. This is probably due to the small quantity of the virus, and to its slower rate of increase.

8. Inferior or imperfect materials for vaccination are more powerful in their action. Thus opaque vaccine and variolous lymph not unfrequently produced eruptions, with a modified imperfect local effect. Cultivation of clear lymph in solid media appears to increase its potency, as shown by its production of an eruptive fever in a calf. Cultivation of vesicular variolous lymph in solid media does not appear to differ in potency from vaccine cultivations, as shown by the effect of inoculation on a monkey. The action of the material containing spores is probably milder.

9. Vaccination probably protects from smallpox, by producing a mild form of fermentation in the blood.

The process probably takes place slowly, as the ferment is growing in unsuitable soil.

10. When the spores or mature forms are inhaled they probably grow in the air-passages with extreme rapidity, and thus get into the blood, where they multiply during the incubative period.

11. My observations appear to show that what is called "attenuation of a virus" may be explained by spore-production. Are not the perfect vaccine materials for infective diseases to be found in the spores of the micro-organisms which are their exciting causes?

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APPENDIX B.

At the Council Chamber, Whitehall, the 1st day of December 1859, by the Lords of Her Majesty's Most Honourable Privy Council.

To the Guardians of the Poor of all Unions and Parishes, to the Wardens and Overseers of all Parishes, Townships, and places in which the Relief to the Poor is not administered by Guardians, in England and Wales, and to all Medical Practitioners.

WHEREAS by the Public Health Act, 1858, and by an Act since passed to perpetuate the same, it is enacted that the Privy Council may from time to time issue such regulations as they think fit, for securing the due qualification of persons to be thereafter contracted with by Guardians and Overseers of Unions and Parishes in England, for the vaccination of persons resident in such unions and parishes, and for securing the efficient performance of vaccination by the persons already or thereafter to be contracted with as aforesaid :—

Now, therefore, it is hereby ordered, by the Lords and others of Her Majesty's Most Honourable Privy Council (of whom the Vice-President of the Committee of the said Privy Council on Education is one) that on and after the 1st day of January 1860, the following regulations shall be in force, viz. :—

1. Except where the Privy Council, for reasons brought to their notice, see fit in particular cases otherwise to allow, no person shall in future be admitted as a Contractor for Vaccination, unless he possess the same qualifications as are

required by the Orders of the Poor Law Commissioners as qualifications for a District Medical Officer, and produce a special certificate, given, under such conditions as the Privy Council from time to time fix, by some public Vaccinator whom the Privy Council authorise to act for the purpose, and by whom he has been duly instructed or examined in the practice of vaccination, and all that relates thereto :—

But the production of this special certificate on occasion of the contract being made may be dispensed with if the certificate, or some other which the Privy Council judge to be of like effect, have been among the certificates, or testimonials necessary for obtaining any diploma, licence, or degree, which the candidate possesses ;

And also, in respect of persons legally admitted to practice before this regulation comes into effect, the special certificate may be dispensed with, on condition that the contract, during one year from its making, continue subject to the approval of the Poor Law Board ;

And all persons now contracted with shall be deemed to be qualified to be again contracted with.

2. Under the same conditions as are appointed for the admission of a contractor, any person qualified to be a contractor may, on the contractor's application, be admitted by the guardians or overseers to act as his occasional deputy ;

But, if this admission be not part of the original contract, it must be notified by indorsement upon the contract ; and at least 15 days before it is intended to take effect, a copy of the proposed endorsement, together with all requisite evidence of the qualification of the person whom it is proposed to admit, must be transmitted to the Poor Law Board.

3. All vaccinations and inspections under contract shall be performed by the contractor in person, or by some other

contractor of the same union or parish acting for him, or by a deputy, duly admitted as above:

But, at any station where the contractor is authorised (as above) to grant certificates, pupils and other candidates, aged not less than 18 years, may, in his presence and under his direction, take part in vaccinating.

All vaccinations and inspections under contract shall be performed in accordance with the annexed "Instructions for Vaccinators under Contract."[1]

4. Until some new form of Vaccination Register be duly prescribed, the person who performs any vaccination under contract, shall, on the day when he performs it, legibly write in his register (as now provided) the letter R (for re-vaccination) against the name of every person, adult or adolescent, who, having in early life been successfully vaccinated, is re-vaccinated; and shall also enter in some column, or in the margin of the register, the source whence the lymph used in the vaccination was obtained;—

Thus: the name, or number (if any) in the register, of the subject from whom the lymph was taken; or "N.V.E.," if the lymph was sent by the National Vaccine Establishment; or the name or description of any other source;—

And where the vaccination or the inspection is done by a person acting as deputy for the contractor, the deputy shall write the initials of his name in the register side by side with the entry of the case; viz., in the left margin of the page, if it be a vaccination which he performs, or in the right margin of the page, if it be an inspection which he performs.

5. Guardians and overseers, in their respective unions and parishes, shall forthwith take measures to bring the performance of public vaccination into conformity with these regulations.

WM. L. BATHURST.

[1] For these instructions, see annexed order of February 28, 1887.

APPENDIX C.

To the Guardians of the Poor of the several Unions and separate Parishes in England and Wales; and to all others whom it may concern.

Whereas by an Order of the Lords of Her Majesty's Most Honourable Privy Council, dated the 29th day of July 1871, it was provided that all vaccinations and inspections under Contract should be performed in accordance with the "Instructions for Vaccinators under Contract" annexed to such Order:

Now, therefore, We, the Local Government Board, in pursuance of the powers given to Us by the Statutes in that behalf, hereby Order as follows:—

Article I.—The said Order shall be rescinded from and after the Fifteenth day of March, 1887, except so far as it rescinded certain provisions of an Order of Her Majesty's Most Honourable Privy Council, dated the 1st day of December, 1859.

Article II.—All vaccinations and inspections under Contract shall be performed in accordance with the "Instructions for Vaccinators under Contract" contained in the Schedule appended to this Order.

Article III.—This Order shall come into force and have effect on and after the Fifteenth day of March, 1887.

Article IV.—In this Order,—

The word "Union" includes any Union of Parishes incorporated or united for the relief or maintenance of the Poor under any Act of Parliament;

The term "Separate Parish" means a Parish or Place which is under a separate Board of Guardians;

The word "Guardians" includes any Governors, Directors, Managers, Acting Guardians, Vestrymen, or other Officers appointed or entitled to act in the distribution or ordering of relief to the Poor from the Poor Rates under any Act of Parliament.

Given under the Seal of Office of the Local Government Board, this Twenty-eighth day of February, in the year One thousand eight hundred and eighty seven.

CHAS. J. RITCHIE,
President.

S. B. PROVIS,
Assistant Secretary.

INSTRUCTIONS FOR VACCINATORS UNDER CONTRACT.

(1) Except so far as any immediate danger of smallpox may require, vaccinate only subjects who are in good health. As regards infants, ascertain that there is not any febrile state, nor any irritation of the bowels, nor any unhealthy state of skin; especially no chafing or eczema behind the ears, or in the groin, or elsewhere in folds of skin. Do not, except of necessity, vaccinate in cases where there has been recent exposure to the infection of measles or scarlatina, nor where erysipelas is prevailing in or about the place of residence.

(2) In all ordinary cases of primary vaccination make

sueh insertions of lymph as will produce at least four separate good-sized vesieles or groups of vesieles, not less than half an ineh from one another. The total area of vesieulation on the same day in the week following the vaecination should not be less than half a square ineh.

(3) Direet that eare be taken for keeping the vesieles uninjured during their progress, and for avoiding afterwards the premature removal of the erusts. Do not use any needless means of "protection" or of "dressing" to a vaeeinated arm; but if in a partieular ease you find reason for means of "proteetion" or of "dressing," define the material and the manner of use of the applianee best adapted to the ease, avoiding all sueh as eannot readily be destroyed and replaeed whenever they beeome soiled.

(4) Enter all eases in your Register on the day when you vaeeinate them, and with all partieulars required in the Register up to and including the column headed "Initials of Person performing the Operation." Enter the results on the day of inspeetion. Eaeh of those entries must be attested by the initials of the person who inspeets the ease. In eases of primary vaeeination, register as "sueeessful" only those cases in whieh the normal vaceine vesiele has been produced; in eases of re-vaeeination, register as "suceessful" only those cases in whieh either vesieles, normal or modified, or papules surrounded by areolæ, have resulted. When any operation (whether vaeeination or re-vaeeination) has to be repeated owing to want of sueeess in the first instanee, it should be entered as a fresh ease in the Register.

(5) Endeavour to maintain in your district such a succession of eases as will enable you to vaceinate with liquid lymph direetly from arm to arm at eaeh of your Contract attendances; and do not, under ordinary circumstanees,

adopt any other method of vaccinating. To provide against emergencies, always have in reserve some stored lymph; either *dry*, on ivory points, thickly charged and constantly well protected from damp; or *liquid*, in fine, short, uniformly capillary (not bulbed) tubes, hermetically sealed at both extremities. Lymph, successfully preserved by either of these methods, may be used without definite restrictions as to time. With all stored lymph caution is necessary, lest in time it have become inert, or otherwise unfit for use.

(6) Consider yourself strictly responsible for the quality of whatever lymph you use or furnish for vaccination. Never either use or furnish lymph which has in it any, even the slightest, admixture of blood. In storing lymph, be careful to keep separate the charges obtained from different subjects, and to affix to each set of charges the name, or the number in your Register, of the subject from whom the lymph was derived. Keep such note of all supplies of lymph which you use or furnish as will always enable you to identify the origin of the lymph. Do not employ lymph supplied by any person who does not keep exact record of its source.

(7) Never take lymph from cases of re-vaccination. Take lymph only from subjects who are in good health, and, as far as you can ascertain, of healthy parentage; preferring children whose families are known to you, and who have elder brothers or sisters of undoubted healthiness. Always carefully examine the subject as to any existing skin disease, and especially as to any signs of hereditary syphilis. Do not take lymph from children who have any sort of sore at or about the anus. Take lymph only from well characterised, uninjured vesicles. Take it at the stage when the vesicles are fully formed and plump. Do not take it from a vesicle around which there is any conspicuous commencement of

areola. Open the vesicles with scrupulous care to avoid drawing blood. Take no lymph which, as it issues from the vesicle, is not perfectly clear and transparent, or which is thin and watery. From a well-formed vesicle of ordinary size do not, except under circumstances of necessity, take more lymph than will suffice for the immediate vaccination of five subjects, or for the charging of seven ivory points, or for the filling of three capillary tubes; and from larger or smaller vesicles, take only in like proportion to their size. Never squeeze or scrape or drain any vesicle, and do not use lymph that has run down the skin. Be careful never to transfer blood from the subject you vaccinate to the subject from whom you take lymph.

(8) Scrupulously observe in your inspections every sign which tests the efficiency and purity of your lymph. Note any case wherein the vaccine vesicle is unduly hastened or otherwise irregular in its development, or wherein any undue local irritation arises; and if similar results ensue in other cases vaccinated with the same lymph, desist at once from employing it. Consider that your lymph ought to be changed if your cases, at the usual time of inspection on the day week after vaccination, show any conspicuous areolæ round their vesicles.

(9) Keep in good condition the lancets or other instruments which you use for vaccinating, and do not use them for any other purpose whatever. When you vaccinate, have water and a napkin at your side, with which invariably to cleanse your instrument after one operation before proceeding to another. Never use an ivory point or capillary tube a second time either for the conveyance or for the storage of lymph, but when points or tubes have once been charged with lymph and put to their proper use, do not fail to break or otherwise destroy them.

N.B.—Supplies of lymph are furnished to medical practitioners on personal application at the New Government Offices, Whitehall, London, S.W., between the hours of 12 and 2; or by letter addressed as follows:—

To
The Secretary,
Local Government Board,
Whitehall,
London, S. W.

National Vaccine
Establishment.

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